Complexity, unpredictability and safety challenges of lipid nanoparticles - A multidisciplinary narrative review

L.Maria. Gutschi BScPhm, PharmD[1] & Falko Seger[2]

[1] **Pharmacy Consultant, Independent Researcher, Ottawa, Canada ORCID 0009-0008-0020-0826**

[2] **Independent Researcher, Berlin, Germany**

Abstract

The lipid nanoparticle (LNP) platform for delivering modified messenger RNA (modRNA) represents a transformative yet inherently complex and unpredictable technology. This narrative review synthesizes multidisciplinary evidence to explore the physicochemical basis, biological interactions, pharmacodynamic uncertainties, and safety challenges associated with LNPs and LNP-modRNA interactions. We describe how LNP self-assembly gives rise to variable structures with inconsistent modRNA payloads, as well as dynamic protein corona formation and aggregation phenomena that complicate the reliable characterization of these systems. After injection, LNPs undergo rapid biotransformation, including PEG-lipid shedding, biodistribution, and cellular uptake, which current analytical techniques cannot fully capture.

Importantly, endosomal escape, which leads to the disruption of the endosome and the release of the payload, occurs within a narrow time window, is often inefficient, and results in inconsistent delivery. In addition, lipid metabolites, cell membrane modulation, and adduct formation pose poorly characterized risks.

Keywords: lipid nanoparticles, mRNA vaccines, protein corona, endosomal escape, unpredictability, drug interactions, safety

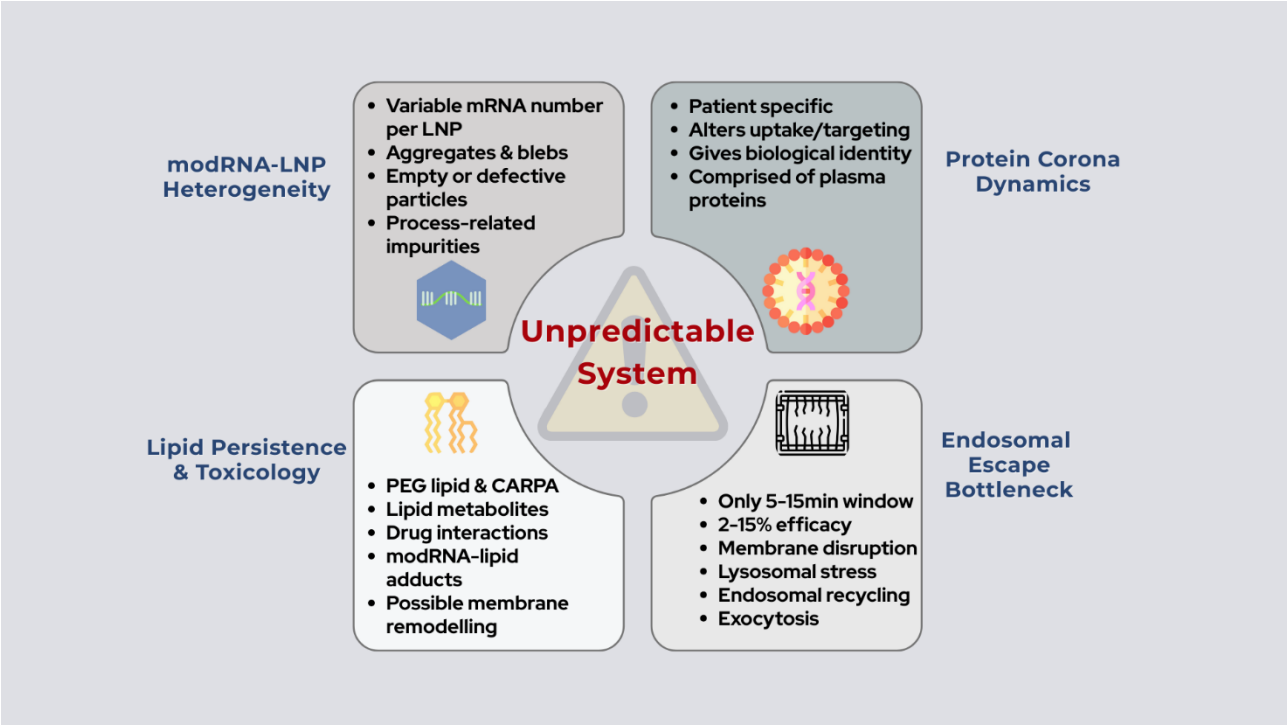


Figure 1

Conceptual overview of the unpredictable LNP platform. Four key challenges are highlighted. 1. LNP heterogeneity (variable modRNA content, aggregates, impurities) 2. Protein corona dynamics (patient-specific, uptake, biological identity) 3. Lipid persistence and toxicology (PEG lipid immunogenicity, modRNA-lipid adducts) and 4. Endosomal escape bottleneck (5-15min window, low efficacy, membrane disruption) Original work using Canva by S. Natsheh. Icons made by Pixel perfect from www.flaticon.com

1 Physicochemical Foundations of the LNPs

1.1 Introduction

The physicochemical properties of lipid nanoparticles (LNPs), including their size, shape, surface reactivity, and lipid composition, are crucial for their role in delivering modRNA to cells. These in vitro properties govern LNP stability, encapsulation efficiency, and the ability to penetrate the cell membrane and transport the modRNA into the cytosol. The physicochemical properties of LNPs profoundly affect the lipid chemistry of the cell membrane, which varies between different cells and cell types. This is important since the membrane is inherently connected to the intracellular signal transduction

network, which is initiated and regulated by endocytic processes and receptor conformational changes, many of which depend on the physicochemical properties of the LNPs. This section thoroughly investigates the LNP composition, structure, and nanoparticle characteristics, establishing a foundation for understanding their behavior in vivo.

LNPs are by no means new.(Cullis & Felgner, 2024; Tenchov et al., 2021) Research into lipid carrier systems with a wide variety of formulations has been ongoing for over 60 years. Liposomes are an earlier type of LNPs, consisting of one or multiple lipid bilayers with an aqueous core. They are commonly used in drug delivery because hydrophilic drugs can be enclosed within the aqueous interior, while hydrophobic drugs are trapped within the hydrocarbon chains of the lipid bilayer. Liposomes cannot efficiently carry nucleic acids, such as mRNA, due to the size, polyanionic nature, and hydrophilicity of the mRNA, which motivated the development of ionizable lipid-based LNPs. Additionally, nucleic acids are quickly degraded by endogenous nucleases in bodily fluids.(Kloczewiak et al., 2022) To address these issues, LNPs incorporating ionizable lipids have been developed as delivery vehicles for small interfering RNA (siRNA) and mRNA, thereby protecting fragile cargo from degradation in vivo and facilitating cellular delivery.

Despite their widespread clinical application in SARS-CoV-2 vaccination, the complex multicomponent nature of LNP systems leads to heterogeneity and unpredictability at multiple levels of biological interaction. Regulatory assessments have traditionally categorized LNPs as inert excipients, but accumulating evidence points to adjuvant-like properties, complement activation, immunomodulation, and potential drug–vaccine interactions caused by cytokine-mediated suppression of cytochrome P450 enzymes. Taken together, these findings suggest that LNPs should be regarded as active pharmacological entities rather than passive carriers, whose systemic and long-term effects remain incompletely understood.

While prior reviews have explored the properties of LNPs(Tenchov et al., 2021) or safety aspects,(Bitounis et al., 2024) the present work represents a first attempt to integrate the unpredictable and partially stochastic nature of modRNA–LNP systems across their pharmacological dimensions.

We argue that this non-linear behavior introduces uncertainty into therapeutic application and challenges precision and predictability. Accordingly, we emphasize the need for enhanced regulatory oversight, thorough mechanistic studies, clinical pharmacology assessments, and the application of advanced analytical techniques to better characterize and evaluate this novel platform.

1.2 Composition

The currently approved LNP formulations for the COVID-19 vaccines contain four lipids: (1) an ionizable cationic lipid, (2) a helper lipid DSPC (1,2-distearoyl-*sn*-glycero-3-phosphocholine), (3) cholesterol, and (4) a polyethylene glycol (PEG)-lipid conjugate.(Chaudhary et al., 2024) Each lipid component of the nanoparticle and its molar ratio are critical to the activity and disposition of the modRNA. Similarly, the first approved LNP-RNA product, patisiran (Onpattro), contains short interfering RNA (siRNA) in an LNP formulation designed to deliver siRNA to the liver and silence the expression of transthyretin, a protein that causes transthyretin amyloidosis (ATTR).

Developing and scaling up Onpattro® paved the way for LNP-modRNA vaccines, which are the fastest vaccines ever produced.(Hald Albertsen et al., 2022)

The ionizable lipid is crucial for delivering nucleic acids across cell membranes. Composed of a tertiary amine head, a linker, and a hydrophobic tail, it undergoes protonation under acidic conditions. This allows it to bind to negatively charged modRNAs, specifically via the tertiary amine head, owing to the unique properties and pH-dependent surface charge of ionizable lipids (Han et al., 2021). The design of the ionizable lipid, such as tail length(Hashiba, Taguchi, Sakamoto, Otsu, Maeda, Suzuki, et al., 2024), saturation, and branched tails, (Petersen et al., 2024) influences the efficacy and toxicity of the LNPs. The helper phospholipid (DSPC) enhances LNP bilayer stability, thereby preventing leakage of nucleic acid cargo. It provides the structural foundation for membrane fusion, which is necessary for cellular uptake. Cholesterol is crucial for maintaining the overall shape, fluidity, and permeability of the bilayer membrane, as well as supporting other phospholipids for effective encapsulation and protection of the

modRNA cargo,(Wang et al., 2024). Cholesterol accounts for about 45% of the LNP content and can exist in a crystalline-like state within the LNP.(Anindita et al., 2024)

The PEG lipid conjugate serves primarily to decrease LNP size, shield the LNP from rapid clearance by the reticuloendothelial system (RES), stabilize LNPs via steric repulsion, and prevent protein adsorption due to the hydrophilic chains extending from the surface.(Hald Albertsen et al., 2022) It typically only comprises about 1.5% of the LNP content. The immunogenicity of PEG has drawn attention due to the development of anti-PEG antibodies after repeated exposure.(Song et al., 2025)

1.3 Structure of the LNPs

For COVID-19 vaccines, the exact structures of modRNA-LNPs remain unknown due to their self-assembly nature and the properties of the lipids used. These Janus particles, which exhibit two or more distinct physical properties, remain poorly understood. Small-angle neutron scattering (SANS) reveals that blebs (separate aqueous-filled compartment within a lipid nanoparticle, distinct from the main lipid structure) are common, but they do not always indicate the presence of modRNA within them.(Chen et al., 2025) In fact, identifying modRNA-free LNPs has proved particularly challenging. Studies estimate that 12-80% of LNPs (most recently 30-35%) may lack any modRNA, depending on the manufacturing process, the ionizable lipid used, and the analytical method employed.

(Li et al., 2022; Münter et al., 2024; Pavlin et al., 2025; Schober et al., 2024) The modRNA payload is especially important, particularly regarding the number of strands and the structure of the modRNA, as the random packaging of modRNA constructs influences LNP behaviour and potency.(Liao et al., 2025) (Renzi et al., 2024) (Di et al., 2022) Therefore, the relationship between the declared dose (µg of RNA) and the number of RNA-containing particles is not straightforward, and this correlation has yet to be fully described.

Currently, there is no reliable analytical method to accurately characterize either the content (i.e., the modRNA,(Webb et al., 2025)) or the structure of LNPs(Sanyal et al., 2021), so orthogonal techniques are necessary.(Parot et al., 2024; Pavlin et al., 2025) Moreover, LNPs with blebs may also exhibit different immunogenicity, biodistribution, or *in vivo* properties that have not been adequately studied.(Simonsen, 2024) Mixing and filling parameters during manufacturing and sample handling of filled vials by clinicians also impact modRNA payload.(Matthessen et al., 2024) Furthermore, empty LNPs may reduce the effective dose, increase variability in therapeutic effectiveness since these are the ones most likely to transfect cells,(Liao et al., 2025) and accumulate in tissues possibly acting as adjuvants,(Lee et al., 2023) an understudied risk. These recent findings have raised questions about the formulation and composition of safe and effective LNPs for modRNA therapeutics and makes it difficult to comply with recommendations for LNP characterization by regulatory authorities. (EuropeanMedicinesAgency, 2025) Lyophilization (freeze drying) could reduce empty LNPs and improve stability at room temperature (De & Ko, 2023) and improve mixing, but remains investigational.

1.4 The Nanoparticle Nature of LNPs

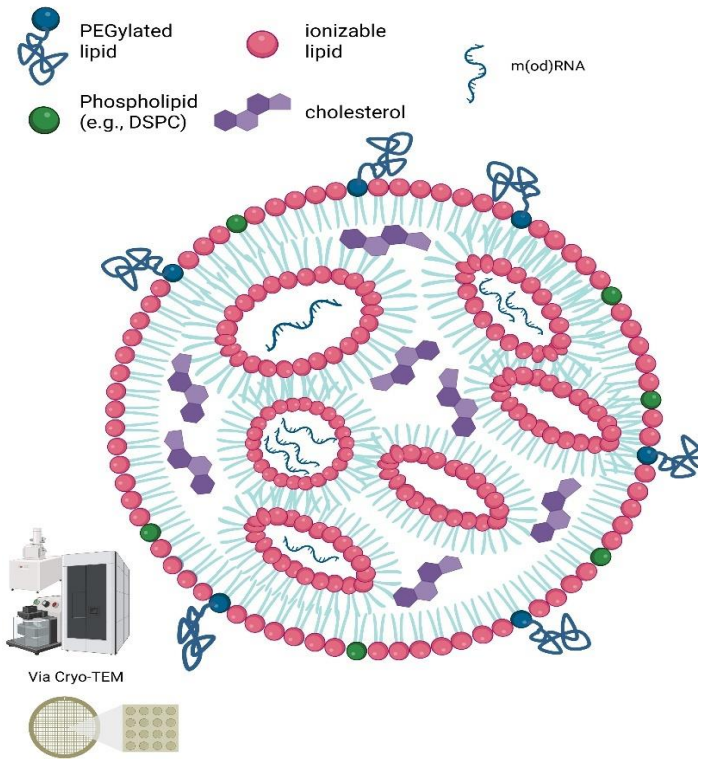
Due to their small size, nanoparticles have an extremely high surface area relative to their volume, resulting in unique chemical, physical, and biological properties not found in bulk materials. These properties enhance the LNPs' reactive interactions with the cell membrane, such as immune responses and cellular uptake.(Yuan et al., 2024)

Importantly, the biological behaviour of the LNP formulation cannot be inferred merely from the isolated properties of individual lipids. This is because the physicochemical characteristics of the entire LNP present in the final formulation, such as size distribution, shape, surface charge or zeta potential, agglomeration state, and lipid packing,(Abbasi et al., 2023) arise from interactions among all the components. For example, the degree of lipid unsaturation and branching affects not only membrane fusion capabilities but also biodegradability and systemic persistence.(Yang et al., 2022) Secondly, variations in size, modRNA payload, encapsulation rate, stability, lipid impurities, and other physicochemical factors may affect the safety and efficacy of these products, as demonstrated in preclinical and clinical studies.(Yuan et al., 2024) (**see Table 1**).

The LNPs are dynamic and unstable

Most recently, evidence suggests that the physiological stability of RNA-LNPs significantly impacts their therapeutic efficacy, pharmacokinetics (PK), tissue-targeting ability, and toxicity.(Zhang & Barz, 2025) Instability in blood or plasma can lead to premature degradation of LNPs and the release of modRNA, potentially altering biodistribution and immune effects or affecting potential inflammation, depending on the specific formulation.(Eygeris et al., 2022)T he Moderna and Pfizer/BioNTech vaccines differ in LNP behaviour. The modRNA of the Moderna COVID-19 vaccine persisted longer in plasma than the ionizable lipid SM-102 itself, suggesting lipid transfer to lipoproteins or extracellular vesicles (EVs).(Kent et al., 2024; Y. Ren et al., 2025). The implications for cellular function remain uncertain. Conversely, the ionizable lipid of Pfizer/BioNTech's vaccine, ALC-0315, showed prolonged lipid exposure but lower levels of modRNA in plasma.(Y. Ren et al., 2025) This could indicate instability of the intact LNP in plasma, possibly caused by trace impurities of the ionizable lipid(Liau et al., 2024) or complete disintegration in plasma(Bitounis et al., 2024) These differences between the approved vaccines suggest that the specific formulation and manufacturing of the modRNA and lipid components (**Figure 2**) are distinct both in composition and biological effects, which may influence vaccine efficacy and outcomes. A comparison of the publicly available compositions, physicochemical properties, and key formulation parameters of the currently approved LNP-RNA products is shown in **Table 1**.

186 **Figure 2** https://BioRender.com/ysmgilk



187

188

Figure 2 Schematic Structure of mRNA-Lipid Nanoparticle

Lipid nanoparticles are mainly composed of ionizable lipids, cholesterol, phospholipids, and polyethylene glycol (PEG)-lipid.

The ionizable lipids are cationic (positively charged) at a low pH (enabling negatively charged RNA complexation) and neutral at physiological pH (reducing potential toxic effects), allowing a better delivery of mRNA into the cells via endocytosis.

Phospholipids play a structural role, and cholesterol serves as a stabilizing element in lipid nanoparticles. Lipid-anchored PEGs dominantly deposit on the lipid nanoparticle surface as a barrier to sterically stabilize them and reduce nonspecific binding to proteins. **Created in BioRender. Seger, F. (2025)**

Category	Pfizer/BioNTech (modRNA)	Moderna (modRNA)
Name	BNT162b2, Comirnaty	mRNA-1273, SpikeVax
Dose, Route	30 μg/0.3 ml, IM	50 μg/0.5 mL, IM
Lipid Components	ALC-0315 (ionizable lipid, Acuitas) ALC-0159 (pegylated lipid) DSPC (neutral lipid) Cholesterol	SM-102 (ionizable lipid) PEG-DMG (pegylated lipid) DSPC (neutral lipid) Cholesterol
Molar Ratios (%) ***(ionizable cationic lipid: neutral lipid: cholesterol: PEGylated lipid)***	46.3:9.4:42.7:1.6	50:10:38.5:1.5
Molar N/P ratios	6	6
Ionizable Lipid Properties	Apparent pKa=6.09 least stable 2 branched chains; moderate biodegradability 2 chiral centres, 3 stereoisomers (De et al., 2025)	Apparent pKa=6.68 more stable 1 branched chain; improved biodegradability No chiral centres
LNP Particle Size and Distribution(Hermosilla et al., 2023**)**	Widest distribution (60-5000nm)	Wider distribution (30-1000nm)
modRNA payload (number of intact modRNA constructs per LNP)	Variable (exact payload unclear)	Variable (exact payload unclear)
Encapsulation Efficiency (%EE)*	~50% (Schober et al., 2024)	Not reported but likely similar
Stability	Moderate (Y. Ren et al., 2025)	High (Y. Ren et al., 2025)
Buffer	Potassium dihydrogen phosphate; Disodium hydrogen phosphate dihydrate pH 7–8; Tris (tromethamine) in October 2021(USFDA, 2021)	Tris (tromethamine) pH 7–8

189

TABLE 1:Composition and Physicochemical Properties of LNPs in Approved modRNA Vaccines (Abstracted from Schoenmaker (Schoenmaker et al., 2021), Zhang, Akinc(Akinc et al., 2019; Zhang et al., 2023); EMA(EMA/707383/, 2020 Corr.1*[1]),(EuopeanMedicinesAgency, 2021))

***United States Pharmacopeia** uses EE(%), defined as the percentage of RNA or therapeutic cargo that is successfully enclosed within the LNPs relative to the total amount of RNA present in the final sample. Schober et al(Schober et al., 2024) used encapsulation efficiency as the percentage of input RNA encapsulated in the final LNP product (EE_{input}%) and found encapsulation rates <50%*

1.5 Analytical Challenges and Knowledge Gaps

The physicochemical properties often differ from theoretical predictions based on behaviors observed in non-biological systems. Despite significant progress, reliable techniques to determine physicochemical attributes are not yet fully standardized.(UnitedStatesPharmacopeia, 2024) For instance, particle size varies.(Hermosilla et al., 2023) Using both expired and unexpired batches of BNT162b2 (Comirnaty®) and m-1273 (Spikevax®), the authors identified three different populations of LNPs for Comirnaty®: 60–65 nm (90% of the total), 600–700 nm (5–10%), and, in two vials examined, 5000 nm (1.2% and 2.8% by volume). Similar results were observed for SpikeVax®, ranging from 30 nm to 1000 nm. These large particles likely represent agglomerated LNPs, which are visible particles that may have specific physical, microbiological, and chemical adverse effects.(Liu & Hutchinson, 2024) Aggregates are higher in thawed vials and may have *in vivo* risks (e.g., embolism or inflammation)

These issues complicate accurate assessment of their *in vivo* behaviors, as *in vitro* characterization remains unpredictable and variable.(C. Chen et al., 2023) The need for precise characterization of LNPs, including size, blebs, empty structures, and other parameters, has driven the development of techniques to identify, observe, and measure significant differences between formulations and batch-to-batch variability of the same LNP-RNA system.(Parot et al., 2024) For instance, Pavlin *et al.* (2025) recently introduced a two-dimensional chromatography method that simultaneously assesses encapsulation efficiency (~65–70%), nucleic acid integrity, LNP size and impurities

enabling detection of empty particles and aggregates in heterogeneous samples simultaneously but requires standardization.(Pavlin et al., 2025) The physicochemical and structural complexities, as well as the lack of a reference standard (a certified material for calibration) for LNP formulations(Simon et al., 2023) raise critical questions about their *in vivo* behavior. **Section 2** will expand on this foundation to examine how these properties affect biodistribution, uptake, endosomal escape, therapeutic effectiveness, and potential toxicities.

Section 2 - Biological Interaction and Pharmacodynamic Uncertainties of LNPs

Key Terms in modRNA-LNP Vaccines: Biodistribution, Transfection, and Gene Expression

1. **Biodistribution:** physical location of a drug, tracer, or intact LNP within a biological system.
    a. depends on circulation, the protein corona, vascular permeability, and reticular endothelial system (RES) uptake
    b. does not indicate cell entry
2. **Transfection**: process of delivering nucleic acids, such as modRNA, into eukaryotic cells using nonviral methods.
    a. Requires cellular uptake and endosomal release
3. **Gene expression or Protein Production**
    a. Translation of mRNA into target protein (ie spike protein)
    b. Depends on intact mRNA, active ribosomes, and protection from degradation

Critical Note.

- Biodistribution, transfection and gene expression are time-dependent and distinct processes
- Many studies conflate LNP biodistribution with transfection or gene expression leading to inaccurate assumptions
- Preclinical trials or regulatory submissions often lack transfection and gene expression data, limiting understanding of efficacy and adverse events

2.1 Overview

The *in vivo* journey of modRNA-LNPs from injection to protein translation depends on a variety of interdependent processes. The physicochemical properties, influenced by LNP manufacturing and chemistry, impact the *in vivo* response. This begins with the formation of a protein corona when the LNP interacts with biological fluids. Cell uptake, target cell specificity, reliance on the protein corona, routes of administration, and other factors are not fully captured by current biodistribution analysis methods. Ultimately, endosomal escape releases the modRNA for translation, and the lipids, modRNA, and newly formed protein are cleared and degraded through various pathways. LNP-mediated delivery requires entry into the target cell, traversal of biological barriers and release of modRNA into the cytosol **(Fig. 2**).

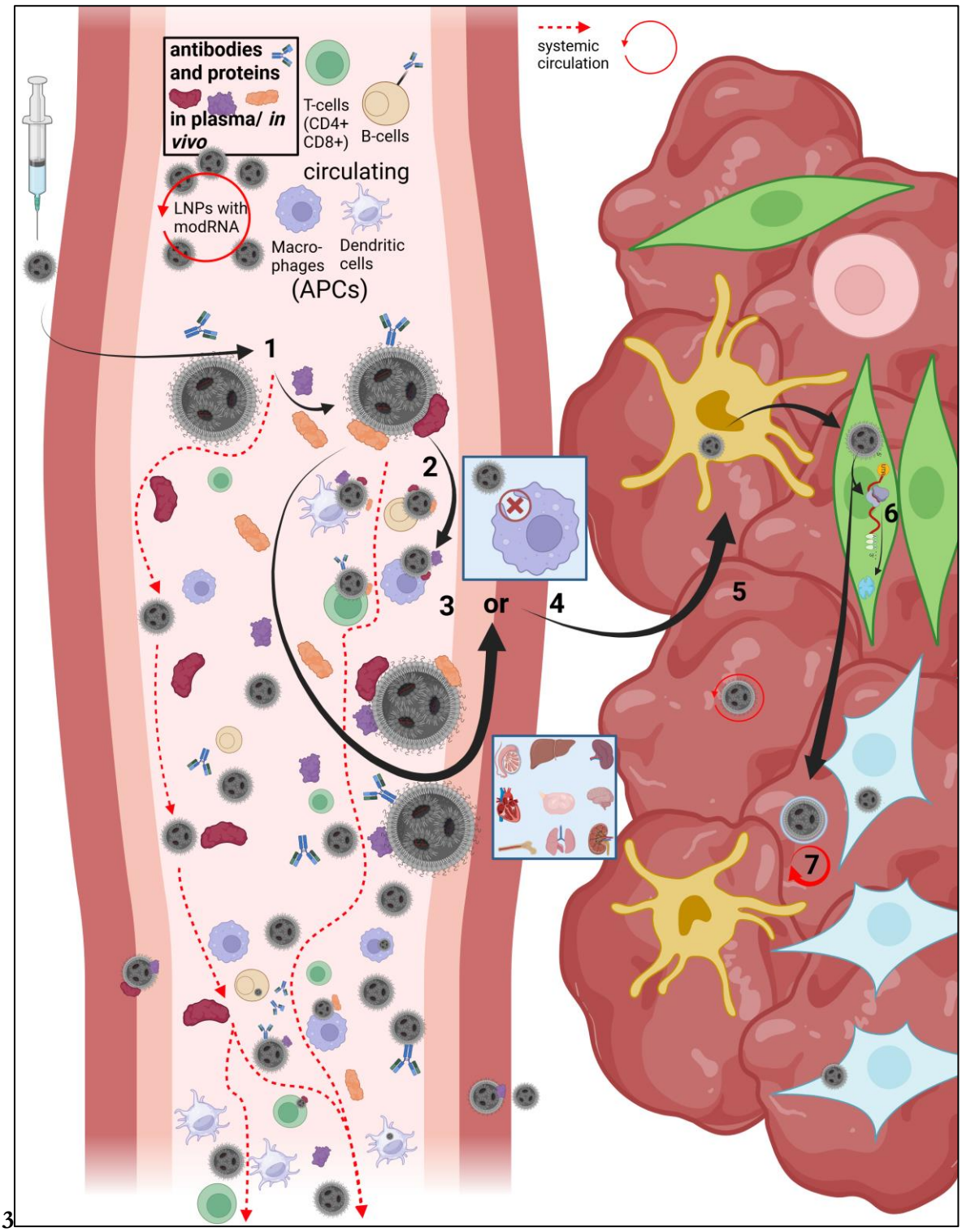


Figure 3
LNP *in vivo* Journey from Injection to Site of Action LNPs injected into muscle rapidly drain into lymph nodes and subsequently circulate in lymph and plasma (the LNP must remain stable in circulation); **2** Acquires an individualized protein corona; **3** Transfects circulating immune cells; **4** Avoids phagocytosis **5** Leaves circulation via fenestrated epithelium or transcytosis **6**. Random transfection of individual cells and release of modRNA into cytosol **7** Exocytosis via extracellular vesicles (EVs)/exosomes. **Created in BioRender. by Seger, F. (2025)**
https://BioRender.com/byxe75h

2.1 Biodistribution of the LNP-modRNA vaccines

Accurately determining the biodistribution of LNPs, the modRNA, and the expressed protein remains a challenge. This issue affects many studies on modRNA-LNP technologies. Fluorescence-based reporter assays are primarily used to track protein production or gene expression, but they do not directly indicate LNP localization or transfection ability. Regulatory guidelines also recommend using quantitative whole-body autoradiography (QWBA) to visualize and quantify intact LNP concentrations across almost all tissues and organs simultaneously, enabling systematic comparisons among tissues.(Vervaeke et al., 2022) Hybridization techniques, such as fluorescence in situ hybridization (FISH) for localization and branched DNA (bDNA) amplification for quantification, are used to study mRNA distribution throughout the body. Luciferase mRNA is a useful reporter for examining biodistribution and protein expression. However, as regulatory authorities note, its short half-life and lack of modifications mean it may not accurately reflect the longer and more sustained protein production typical of modified mRNAs.(EMEA/H/C/005735/RR, 2020) Therefore, conclusions based solely on luciferase mRNA-LNPs may underestimate the actual performance of the modRNA product. These points highlight the complexity of evaluating the biodistribution of modRNA-LNP therapies and emphasize the importance of a layered, comprehensive approach.(Vervaeke et al., 2022)

No biodistribution studies using the actual modRNA from the Pfizer/BioNTech or Moderna vaccine were included in the regulatory documents. As a result, there was no assessment of transfection efficiency or gene expression levels. Further clarification from regulatory authorities and manufacturers is needed to determine the necessary chemical, pharmacological, and toxicological studies for these lipids to obtain approval. (Hemmrich & McNeil, 2023)

Ci *et al.*(Ci et al., 2023) performed one of the few LNP-modRNA biodistribution studies, where the methodology showed strong differentiation of the sequential process of LNP activity based on current technical capabilities. Quantification of the ionizable lipid and its metabolites was accomplished using

LC-MS/MS. ModRNA quantification employed bDNA, and detection of the non-translating Factor IX (NTFIX), a model protein, was analyzed using LC-MS/MS. This multi-faceted analytical approach, performed in mice, allowed for a clear distinction between LNP distribution, modRNA delivery, and downstream protein production. The authors demonstrated both LNP distribution and subsequent protein expression across a wide range of tissues. Protein production was quickly detected in the liver, ovary, and thymus, followed by the uterus and kidneys. As expected, the liver produced the most protein overall, followed by the ovaries, kidneys, and lungs. Protein production persisted at low levels up to 168 hours in the lungs, heart, liver, gastrointestinal tract, kidneys, and uterus, but not in the ovaries; however, no further measurements were obtained. Notably, protein expression was observed in the heart despite little to no corresponding mRNA at later time points, emphasizing the importance of analyzing both mRNA and protein production separately over time to understand the therapeutic effects. These results may indicate that macrophages or dendritic cells traffic to the heart; however, generalizability to humans is unknown. The ionizable lipid and its metabolites were concentrated in the urinary and digestive tracts, suggestive of hepatobiliary and urinary clearance. The ethanolamine portion of the ionizable lipid, radiolabeled with ^{14}C, showed no metabolism in vivo,(Burdette et al., 2023) indicating tissue persistence.

Luo *et al.* (Luo et al., 2025) recently introduced Single Cell Precision Nanocarrier Identification (SCP-Nano), a novel imaging and deep learning pipeline for single-cell resolution mapping fluorescence-labelled carriers such as LNPs across whole mouse bodies at doses as low as 0.0005 mg/kg, which are typical for modRNA vaccines and are 100-1000 times lower than conventional imaging methods, such as QWBA. Using reporter mRNA (e.g. EGFR), the study demonstrated heterogeneous nanocarrier uptake and protein expression both within and across organs, with hotspots in the liver and spleen. This punctuated pattern indicated that some cells successfully translated the mRNA, while neighboring cells exhibited uptake without expression. Intramuscular injection of LNPs with SARS-CoV-2 spike modRNA revealed low-level heart endothelial delivery, confirming possible molecular and proteomic changes beyond primary targets. These results in rodent models may not directly apply to humans. Still, this may have important implications for potential off-target effects that standard diagnostic methods, such as ultrasound or CT scans, might miss. Cellular or molecular changes could contribute to symptoms or disease risk, but may not be visible until they become extensive enough to be detected by conventional tools.

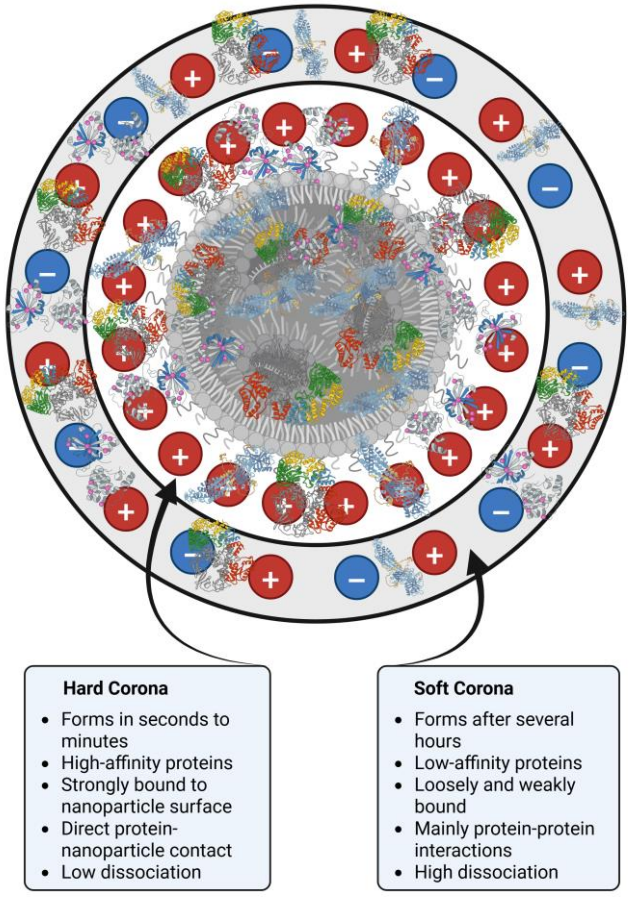
The route of administration also influences the biodistribution of LNP-modRNA therapy. For intramuscular administration, such as that for the modRNA COVID-19 vaccines, syringe pressure, perfusion rate, proximity to blood vessels and lymphatic vessels, local pH, and temperature, among others, are important considerations. (Naasani, 2022) In contrast, other LNP-nucleic acid therapies, such as the siRNA product patisiran (Onpattro®), are administered through intravenous infusion, which achieves liver targeting almost exclusively through ApoE binding and LDL receptor uptake. Subcutaneous and intranasal routes favour lymph nodes and lungs, respectively.(Mendonça et al., 2023) These differences demonstrate that the biodistribution of LNPs differs significantly based on the route of administration, making them distinct from traditional small-molecule therapeutics.

2.3 Formation and Biological Role of the Protein Corona

When LNPs encounter biological fluids, they are immediately transformed by their environment. They acquire a dynamic and heterogeneous coating of biomolecules known as the protein corona. This layer fundamentally changes how the body perceives and processes the LNPs, influencing biodistribution, immune recognition, cellular uptake, and ultimately the efficiency of modRNA translation. Therefore, the protein corona gives the LNPs a "biological identity".(Akhter et al., 2021)

The protein corona formation occurs within minutes through van der Waals forces, hydrophobic interactions, electrostatic interactions, and other biochemical and biophysical interactions, resulting in an individual, heterogeneous *in vivo* LNP pool.(Cedervall et al., 2007) For the modRNA-LNPs, this process is accelerated because the PEG-lipids on the surface of the modRNA-LNPs dissociate and exchange with plasma proteins, a mechanism known as PEG-lipid shedding.(Escalona-Rayo et al., 2024) It is a dynamic, complex, and unpredictable process that is crucial for biodistribution, transfection, and cellular responses, since this is what the cell itself "sees."(Walczyk et al., 2010)

336 **Figure 4:The Biocorona Created in BioRender. Seger, F. (2025)** https://BioRender.com/edw2fru



337

Composition and Determinants of the Biocorona

Composition

- Lipoproteins, immunoglobulins, albumin, complement, etc
- Species-specific, impact on animal models for toxicity studies

Influencing Factors

- Physicochemical properties (size, shape PEG-lipid density, shedding rate
- Environmental factors; temperature, pH, incubation time, biological fluid (plasma, lymph), age, gender, comorbidities

Human biocorona characterization

- Not fully characterized
- LNP size and density resemble natural serum lipoproteins
- Voke *et al* found consistent proteins, i.e.ApoE, C-reactive protein, alpha-2 macroglobulin, vitronectin

Dynamic Remodelling

- Biocorona changes as the LNP moves through biological fluids, hard and soft corona
- Complicates LNP behaviour prediction

The biocorona can alter the internal structure of the LNPs.

The protein corona can potentially mask targeting ligands or alter interactions with cell membranes. This can reduce the efficacy of targeted delivery by shielding functional moieties or, in some cases, enhance functionality by presenting a new protein-based signal.(Voke et al., 2025)

One of the most critical aspects of the protein corona was demonstrated by Sebastiani *et al.*(Sebastiani et al., 2021) When ApoE binds to the protein corona of LNPs, the entire biodistribution pattern of the original formulation is altered by internal structural changes, potentially affecting modRNA encapsulation, agglomeration and premature RNA release. Accordingly, the entire surface structure changes, facilitating the opsonization of phagocytes, such as macrophages and dendritic cells. Further work also emphasized the importance of the protein corona for not only biodistribution but also transfection efficiency and translation yield. (da Costa Marques et al., 2023; Huang et al., 2023; K. Liu et al., 2023; Sengottiyan et al., 2023)

The immunological effects of the biocorona in plasma

The accelerated blood clearance (ABC) phenomenon, often triggered by repeated administration of PEGylated LNPs, results from the production of anti-PEG antibodies. These antibodies can quickly clear subsequent doses of PEGylated LNPs from the bloodstream through accelerated blood clearance, reducing therapeutic effectiveness but also potentially increasing the risk of adverse reactions due to the rapid and unpredictable distribution of the nanoparticles.(Wang et al., 2024) PEGylated nanoparticles are known to interact with circulating complement proteins, activating the complement cascade and producing opsonins and anaphylatoxins, which are associated with acute infusion reactions in patients, known as complement activation-related pseudoallergy (CARPA).(Szebeni et al., 2018) Anaphylactic and allergic reactions observed after modRNA COVID-19 vaccination may partly reflect this phenomenon.(Bakos et al., 2024)

Implications and Challenges

Since processing and administration into a living organism involve many interfering factors, it seems plausible that the biocorona causes a nonlinear distribution route depending on the formulation of the LNPs, the biological environment, and the route of administration. Overall, these data challenge the idea of a uniform LNP formulation and predictable biodistribution. One might assume that if these factors heavily influence biodistribution, administering the same dose to two subjects is unlikely to result in similar responses. Recent approaches to addressing the inherent issues with the biocorona of LNPs have utilized liposomal LNP-modRNA nanoparticles, which exhibit extra-hepatic targeting and longer circulation lifetimes, likely due to the formation of fewer proteins in the protein corona.(M. H. Y. Cheng et al., 2025) This represents a return to the original nanosized lipid particles, liposomes.

2.4 Target Sites and Tissues

What are the main sites and tissues targeted by the LNPs? The primary target sites for the LNPs include the liver, spleen, and draining lymph nodes, as these organs comprise a significant portion of the Reticuloendothelial System (RES), a component of the immune system that involves phagocytes, such as macrophages and monocytes. These cells are primarily located on the vascular wall of the liver (Kupffer cells), spleen (splenic macrophages), kidneys (mesangial cells), and lungs (lung macrophages).(Ngo et al., 2022) Given the dynamic nature of the biocorona and the common presence of ApoE in it, it is not surprising that hepatocytes in the liver are the primary target for the LNPs.(Hosseini-Kharat M, 2025) Additionally, because the liver functions as a biological filter system, LNPs that are up to 200 nm in size tend to undergo fenestration unless specifically engineered otherwise, which helps their uptake into liver sinusoidal endothelial cells (LSECs).(He et al., 2024) Since LNPs first enter through the sinusoidal lumen, Kupffer cells are also the initial targets for transfection.(Hosseini-Kharat M, 2025)

Similarly, LNPs tend to distribute to the spleen due to its sinusoidal endothelium, which facilitates LNP uptake. Depending on the LNP formulation and composition, macrophages and dendritic cells can be targeted, which is essential for the efficacy of modRNA-LNP vaccines.(Haghighi et al., 2024) Based on the proportions, shape, charge, and other factors of the LNP lipid, gene expression or protein production can sometimes exceed levels in the liver.(Hald Albertsen et al., 2022)

Draining lymph nodes are a common target for LNPs. The size of their fenestrations (<200nm) allows the LNPs to migrate through lymphatic channels and be taken up by antigen-presenting cells (APCs).(Hassett et al., 2024) Various modifications, especially surface engineering of the LNPs and other adjustments, improve targeting and retention. Depending on their size and other factors, LNPs can also drain directly into lymph nodes. Additionally, larger particles are transported by APCs (mainly dendritic cells) to different locations, such as the heart, which has been suggested to explain immune reactivity and responses.(Milano et al., 2021)

In addition, other organs may exhibit detectable LNP presence in preclinical studies, sometimes referred to as "off-target effects," which are caused by the physicochemical properties of the LNPs and the resulting protein corona. The heart, lungs, adrenal glands, and ovaries are frequently reported in studies involving rodents and non-human primates (NHP).(TherapeuticGoodsAdministration, 2021)

Transcytosis or direct penetration can occur, allowing LNPs to bypass blood-organ barriers. This is important because LNPs can leave the vasculature and cross the blood-brain barrier (Khare et al., 2023) or the intestinal barrier.(Neves et al., 2016) Zhang *et al.* (Zhang et al., 2024) in a comprehensive review list various target cells, such as epithelial, basal, and endothelial cells, and explain how these are particularly likely to be targeted. Other notable examples include cardiac and skeletal muscle, bone marrow-derived dendritic cells and macrophages, as well as various cell types and tissues. (J. Chen et al., 2023; Dey et al., 2021; Han et al., 2021; Khare et al., 2023; Swingle et al., 2023; Younis et al., 2023; Żak et al., 2023)

Regulatory Gap

In official FDA and EMA documents, the "target cells" believed to be transfected by the LNPs are not specified. Notably, the US FDA mentions transfection(USFDA, 2020) Conversely, the EMA states that the viral protein antigen is expressed in the desired conformation(EuopeanMedicinesAgency, 2021) It is unclear whether both agencies refer to the same process or if the EMA distinguishes between transfection and protein expression, as previously discussed. This lack of clear communication and precise data presentation regarding the modRNA-LNP target cells and delivery, combined with support from public health agencies, (CentresforDiseaseControlandPrevention, 2022) has contributed to the common belief that the vaccine is limited to the deltoid muscle. These misconceptions, have led to a limited understanding of the vaccine's potential for broader use and distribution, leaving safety profiling incomplete.

2. 5 Cellular Uptake Mechanism

As we have seen, the adsorption of biomolecules onto the LNP surface establishes a dynamic biocorona overriding the synthetic nanoparticle design. This identity governs cellular interactions by dictating which membrane receptors are engaged, leading not only to biodistribution patterns but also to endocytic pathways and, consequently, the intracellular fate of the encapsulated modRNA. This membrane uptake into cells is termed endocytosis. The efficiency of uptake is profoundly affected by the biocorona, particle size, shape, and net surface charge.(Hald Albertsen et al., 2022)

Transfection occurs when the LNPs are endocytosed and the modRNA subsequently escapes the endosome into the cytosol (**Figure 5**).

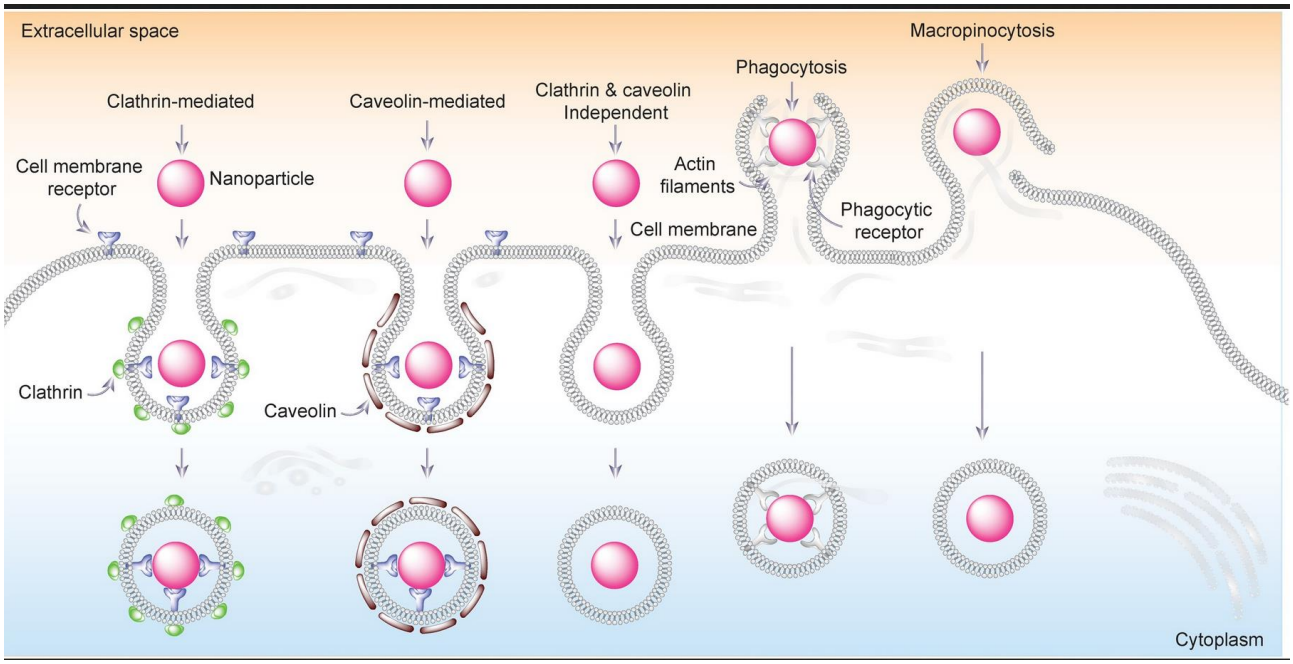


Figure 5. Schematic representation of nanoparticle cellular internalization pathways, including clathrin-mediated, caveolin-mediated, clathrin- and caveolin-independent, phagocytosis, and macropinocytosis. Adapted from Augustine R, Hasan A, Primavera R, et al. Materials Today Communications (2020) 25:101692. https://doi.org/10.1016/j.mtcomm.2020.101692. Licensed under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

Mechanisms of Uptake

There is little focus on how LNPs penetrate the cell membrane, or which receptors and ligands are most likely to interact with LNPs for uptake into cells or endocytosis. In 2008, Lonez et al (Lonez et al., 2008) stated that it was unclear whether receptor-dependent or receptor-independent "endocytosis-like" uptake of liposomes into cells was involved. A reassessment by the same authors in 2012 (Lonez et al., 2012) noted that the exact nature of the endocytic vesicles involved in endocytosis or "endocytosis-like" uptake of LNPs was still "a matter of debate." Whether a receptor- or receptor-independent "endocytosis-like" process occurs strongly depends on the protein corona and the state of the cell encountered by the LNP and the local microenvironment(Behzadi et al., 2017) (pH, bradykinin, prostaglandins, etc). Paunovska *et al.* (Paunovska et al., 2022) reported that LNPs can bind to apolipoprotein E and low-density lipoprotein receptors (LDL-R), whereas Chaudhary et al(Chaudhary et al., 2024)

reported that Toll-like receptor (TLR)4 and CD1d can be internalized with the endosome. Both receptor-mediated and receptor-independent cellular uptake(Akhter et al., 2021) likely occur simultaneously within the same cell. Uptake may also occur under specific conditions without direct binding to membrane components; instead, nonspecific hydrophobic or electrostatic interactions ultimately initiate the process. (see **Table 2**)

Measuring how cell receptors bind is challenging.

The current methods used to study the mechanisms by which LNPs interact with the cell membrane often disrupt the natural protein corona composition, making it challenging to identify which cell receptors recognize and bind to LNPs accurately. Identification of the corona proteins is not sufficient because not every protein in the corona can interact with cell receptors, as they may require correct orientation on the nanoparticle surface. Therefore, identifying which epitopes on the biomolecular corona are accessible to cell receptors is essential for determining potential interactions. Likewise, not all exposed proteins can necessarily bind to receptors, especially if there is competition with other proteins with higher affinity for the same receptors. It is, therefore, important to identify which proteins genuinely participate in these interactions.(Aliyandi et al., 2020)

Lipid–membrane interactions can also influence cell membrane receptor activity and thereby contribute to the uptake of lipid nanoparticles (LNPs). As summarized by Lavington & Watts,(Lavington & Watts, 2020) nanodisc and SMA lipid nanoparticle (SMALP) studies demonstrated that specific lipid components (such as helper lipids) modulate the surrounding membrane environment without directly binding to G-protein coupled receptors (GPCR). Such lipid-induced alterations affect GPCR conformation, ligand binding, and signal transduction, supporting functional receptor interactions. The elements of the protein corona, uptake pathways and primary tissues affected are reviewed in **Table 2.**

Biocorona Components	Main Receptors Engaged	Dominant Uptake Pathways	Cell Types Most Affected	Comments/Impact on transfection
Albumin	gp60 (albondin), SPARC, FcRn(Ji et al., 2024)	Caveolae-Mediated Endocytosis(Lonez et al., 2012; L. Ren et al., 2025) Trancytosis	Hepatocytes Endothelial cells Epithelial cells Tumor cells	Can bypass lysosomes, improved cytosol delivery, recycling endosomes Preferred mechanism for modRNA-LNPs
Apolipoproteins (ApoE, ApoB, ApoA-1)	LDL-R LRP-1 SR-B1	Clathrin-Mediated (Sebastiani et al., 2021; Zhang et al., 2024, Borah, 2025 #1697) or Caveolae Mediated Endocytosis	Hepatocytes, Spleen, macrophages Tissues with LDL-R include adrenals, ovaries, testes Neurons(Martins et al., 2024)	Classic receptor-mediated LNP uptake route with ApoE May lead to lysosomal degradation if clathrin-mediated
Vitronectin/ Fibronectin	Integrins (αvβ3, α5β1)	Clathrin or Caveolae-mediated (lipid rafts) (Sousa de Almeida et al., 2021),(Lavington & Watts, 2020)	Endothelial cells Fibroblasts Epithelial cells Tumor and parenchymal cells Heart in murine models (Luo et al., 2025)	Off-target effects Affected by nanoparticle shape; size, etc.
Alpha-2 macroglobulin	LRP1(Yamamoto et al., 2024)	Primarily clathrin-mediated	Hepatocytes, endothelial cells	Traps LNPs for lysosomal degradation(Tomihari et al., 2023) Reduces efficacy
***C-Reactive Protein**	FcγR, C1q	Phagocytosis, complement activation	Macrophages, neutrophils	Complement activation, CARPA Reduces transfection efficiency

***Immunoglobulins(IgG, IgM), Anti-PEG antibodies**	FcγR, FcαR CSF2RB (new finding)(Baimanov et al., 2025)	Phagocytosis (Sousa de Almeida et al., 2021); (Baimanov et al., 2025) Also clathrin-mediated	Uptake by APC when LNPs are opsonized Spleen, macrophages	Leads to lysosomal degradation Triggers immune response (ABC) CSF2RB potential role for CARPA
***Complement proteins (C3b, C4b etc)**	Complement receptors	Phagocytosis Macropinocytosis (Borah et al., 2025; Miao et al., 2020; L. Ren et al., 2025)	Macrophages Neutrophils Dendritic cells	Strongly degradative; opsonization
Direct	TLR4/CD14	TLR4 is internalized along with the forming endosome (promotes lipid-raft formation), (Chaudhary et al., 2024); (Korzun et al., 2023); (Paunovska et al., 2022)	Dendritic cells, macrophages	Initiates cell signalling and immune activation Leads to lysosomal degradation Receptor recycling
Direct	None	Direct Membrane Penetration GPCR interactions (lipid rafts) (Sakurai et al., 2022); (Lavington & Watts, 2020)	Driven by pH and lipid destabilization (small size, specific surface chemistry or external physical forces (e.g., electroporation))	Bypasses endosomal uptake

Table 2 Biocorona, Receptors and Mechanisms of Uptake

αvβ3=integrin alpha-v beta-3; α5β1=alpha-5 beta-1; ABC = accelerated blood clearance; APC = antigen-presenting cells; ApoE = apolipoprotein E**.** C1q=complement C1q component; CSF2RB=colony stimulating factor 2 receptor beta; CR3b=complement receptor 3; FcγR= Fc gamma receptor; FcαR=Fc alpha receptor; GPCR=G-protein-coupled receptor; LDL-R=low-density lipoprotein receptor; LRP-1=low-density lipoprotein receptor protein-1; PEG=polyethylene glycol; SR-B1=scavenger receptor class B Type 1; TLR4=toll-like receptor 4.

*****Opsonins (e.g. CRP, IgGs, complement) act in the vasculature, whereas integrins and others, mediate uptake at the cell membrane.

The main challenge isn't whether transfection occurred, but how much happens and how conditions in systems biology influence this process. According to current knowledge, organ fenestrations and the pKa value mainly determine biodistribution and cellular uptake. The ζ-potential primarily affects

protein corona formation and the likelihood of its formation. (Patel et al., 2021),(Cedervall et al., 2007) Given the numerous mechanisms, cell receptors, and a wide range of cell types, along with cells at different stages of maturation and division within the same lineage, it is not surprising that efforts to systematically target receptor-driven signalling pathways within a highly complex biological system are inherently problematic.

Interestingly, Zelkoski *et al*(Zelkoski et al., 2025) demonstrated in THP-1 cells that ionizable LNPs can activate both TLR4 signalling pathways, the TIRAP/MyD88-NF*κ*B pathway and the T2025RAM/TRIF-IRF pathway, albeit with differences in magnitude and kinetics: NF-*κ*B signalling was rapid and robust, while IRF activation was weaker and delayed. This observation supports the concept that ionizable LNPs, by altering lipid raft dynamics, can induce overlapping but temporally shifted TLR4 signaling responses, diverging from the canonical temporal segregation of these pathways(Kim et al., 2023)(**Table 2**).

2.6 Endosomal Escape as Key Bottleneck

Transfection, as previously discussed, occurs in a receptor-dependent and/or receptor-independent

manner, indicating a bioactive behaviour that extends beyond the traditional pharmacokinetic approach. Transfection is completed when the modRNA escapes the endosome. Assessing how LNPs are metabolized from a traditional pharmacokinetic perspective is challenging because they are not degraded through organ uptake during cell transfection. Instead, endosomal escape and degradation define the entire spectrum of pharmacodynamics.(Ait-Oudhia et al., 2014; He et al., 2019) The classic absorption, distribution, metabolism, and excretion (ADME) pharmacokinetic model does not apply to liposomal or nanoparticle delivery systems.

The Endosomal Escape Mechanism is Based on Biophysical and Chemical Processes

Endosomes consist of a lipid bilayer similar to the cell membrane, which prevents nucleic acid escape as an evolutionary defense against foreign viral RNA entering the cell. For LNPs carrying modRNA, successful endosomal escape is essential for therapeutic action. After endocytosis, the endosomes increase the acid gradient, which protonates the ionizable lipids within the LNPs. For example, ALC-0315 has an apparent pKa value of ~6.09, and SM-102 has a value of ~6.6. This protonation event triggers the rearrangement of lipid molecules into a lamellar phase within the endosomes, promoting membrane destabilization and releasing the payload into the cytosol, a process known as the proton-driven osmotic swelling or the proton sponge effect.(Fell et al., 2025) (Chatterjee et al., 2024) As the pressure rises, the membrane destabilizes and may rupture, releasing its contents into the cytosol. Endosomal damage, as indicated by galectin recruitment, can occur solely from the presence of ionizable lipids and does not require cystolic delivery of the RNA molecule. (Johansson et al., 2025) Lipid geometry facilitates this process. The conical shape of the branched, unsaturated fatty acid chains promotes negative curvature stress within the membrane, increasing destabilization (Petersen et al., 2024) Computational free energy calculations have shown that both ALC-0315 and SM-102 insert into the cell membrane favourably,(Ermilova & Swenson, 2023) suggesting that ionizable lipids in the current LNP-modRNA vaccines embed into the lipid bilayer. Even transient tearing may contribute to escape. Such tearing has been demonstrated with other nanoparticles.(Er-Rafik et al., 2022). Most recently, LNPs were found tethered to the endosomal membrane and associated with membrane destabilization.(Johansson et al., 2025) Finally, Pilkington et al (Pilkington et al., 2021) suggest that LNPs may perturb lipid raft organization, implying that endosomal escape involves not only endocytosis but also broader effects on membrane dynamics. **Figure 5** shows the typical intracellular journey of a modRNA-LNP**.**

Figure 6: Endosomal Escape a The modRNA is introduced into the early endosome after being taken up via clathrin-mediated endocytosis or LDLR as example internalization, which is governed by the biocorona and lipid raft interactions. b The early endosome and protonation of the ionisable lipids. c The disruption of the early endosome and the release of modRNA, impurities, and modRNA-lipid adducts. **d** Meanwhile, a portion of engulfed LNPs are recycled back into the extracellular space as EVs or exosomes. e Another fraction progresses into late endosomes and eventually into lysosomes, where they are degraded. f Endosomal maturation from early to late stages determines the fate of the cargo: either delivery to the lysosome (**e**) or secretion via exosomes, unless the endosome is disrupted (**f**). **Created in BioRender. Seger, F. (2025)**

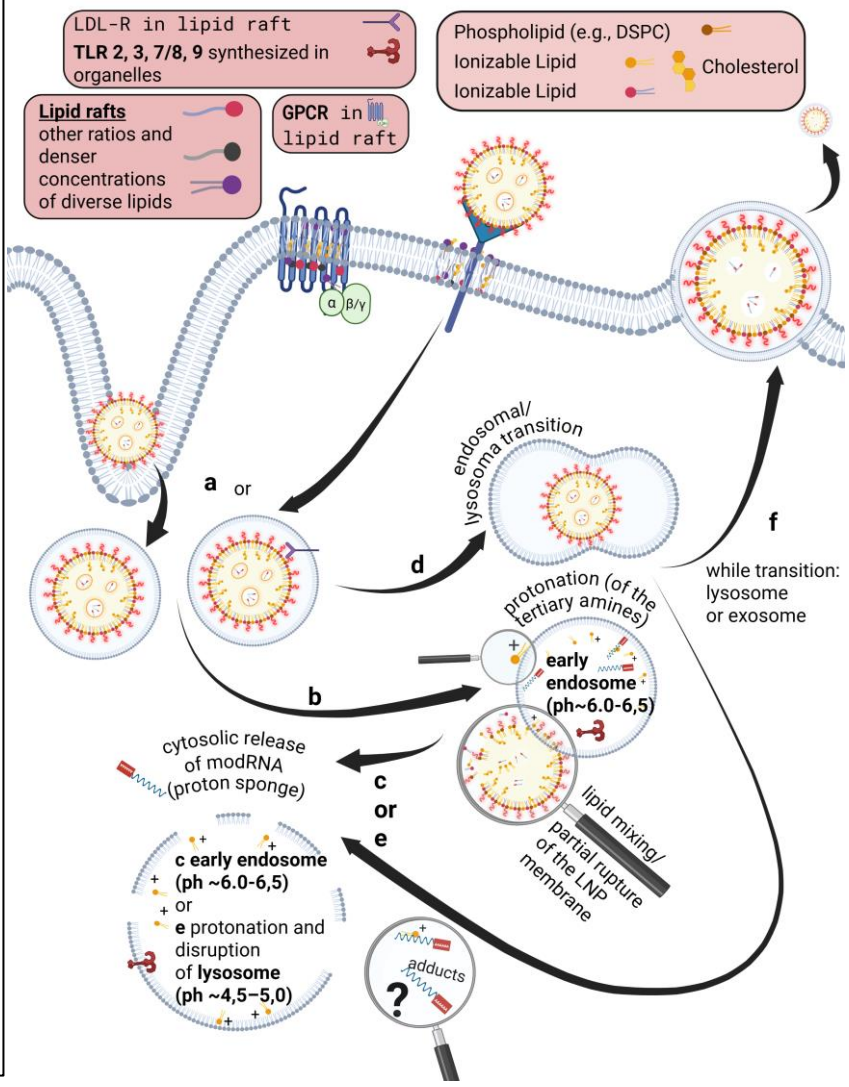


Figure 6 https://BioRender.com/0921yhb

Endosomal Escape is Inefficient

Only within a narrow window of opportunity do conditions allow LNPs to escape through endosomal fusion during the endosomal maturation process.(Chatterjee et al., 2024) (Müller et al., 2024) (Hald Albertsen et al., 2022)

This window is brief, lasting about 5-15 minutes(Schlich et al., 2021) when conditions in the endosome enable the LNPs to fuse with the endosomal membrane and deliver their cargo into the cytosol. Beyond this period, escape efficiency drops significantly.

This process is highly inefficient, with only about 1-15% of all internalized LNPs resulting in the production of the target protein.(Sabnis et al., 2018) (Aliakbarinodehi et al., 2024; Chatterjee et al., 2024)·(Müller et al., 2024) LNPs that do not escape the endosome at this stage are degraded or exocytosed.(Maugeri et al., 2019) Degradation through lysosomal fusion enriches the endosome with degradative contents and enzymes, moving endosomes toward the plasma membrane and enabling fusion for exocytosis. Most LNPs follow these pathways and fail to deliver mRNA to the cytosol, since endosomal escape is the main "bottleneck" of mRNA therapeutics.(Chatterjee et al., 2024). Over the past four decades, numerous methods have been attempted to improve delivery. However, significant improvements in endosomal escape often come at the cost of increased cytotoxicity, such as endosomal bursting and release of entire contents into the cytosol.(Dowdy et al., 2022)

Failure to Escape the Endosomes Results in Cellular Stress

After endocytosis, if the modRNA is not released into the cytoplasm, the endosomes mature into late endosomes and then fuse with lysosomes.(Chatterjee et al., 2024) Lysosomes contain various enzymes such as lipases, proteases, nucleases, and glycosidases that dismantle both the modRNA and lipids. An

accumulation of undegraded materials from the LNPs can trigger cellular stress, oxidative stress, and potential inflammatory signalling. This accumulation has been compared to aspects of lysosomal storage disorders,(Paramasivam et al., 2021)though a direct link to human disease has not been established.

Lysosomal retention blocks expected degradation and recycling processes in the cell, including receptor recycling such as LDL-R. This can create a cellular "traffic jam" that impairs the uptake of new ligands and receptors.(Y. Cheng et al., 2025) Although the lipids comprising the LNPs are considered biodegradable, high local concentrations can impair lysosomal function, slow degradation, and prolong the retention of the disassembled lipids.(Sahay et al., 2013) Consequently, a blockade or arrest of normal endosomal maturation and acidification not only reduces therapeutic efficacy but can also lead to toxicological effects.(Paramasivam et al., 2021)

LNPs May be Expelled Intact or Partially Degraded in Exosomes

Not all LNPs successfully escape the endosomes or are degraded in lysosomes. A significant portion is recycled back into the extracellular space, repackaged in extracellular vesicles (EVs) or exosomes. This pathway enables cells to eliminate undigested LNPs or those that fail to escape the endosomal/lysosomal pathway. Maugeri (Maugeri et al., 2019) showed that LNPs in recycling endosomes are expelled either intact or partially degraded, which affects transfection efficiency. Exocytosis serves as both a clearance route and a secondary distribution mechanism; vesicle-mediated transport may transfer the modRNA or lipid fragments to the surrounding microenvironment in a paracrine manner.(Sahin et al., 2014)

These EVs can also transfect cells, influencing pharmacodynamic outcomes and contributing to variability and off-target effects. In fact, natural exosomes are being engineered for RNA delivery (Iqbal et al., 2024) (Bost et al., 2021) because they can cross physiological barriers effectively, have improved biocompatibility, low toxicity, cell-specific tropism, and can evade the mononuclear phagocytic system. (Wu et al., 2021) This recycling of endosomes, as well as empty LNPs or those with blebs, may cause cellular stress, oxidative damage, and chronic inflammation,(Y. Cheng et al., 2025) which could be linked to adverse effects such as injection-site reactions or immune activation. These factors are not considered in biodistribution studies and may contribute to cumulative toxicity, especially with repeated doses. Long-term studies are needed to determine if these adverse events are causally related, as current regulatory focus is on immediate effects and may overlook these delayed responses.Endosomal escape of siRNA-loaded LNPs, such as those for Onpattro, is minimal, typically around 1%,(Akinc et al., 2019; Dowdy, 2023) which restricts cytosolic delivery and helps minimize cytotoxicity. This low efficiency means that only a small subset of internalized siRNA particles reaches the cytosol. The escape events themselves tend to produce small, transient membrane disruptions that are readily repaired by the cell.(Bates et al., 2025; Johansson et al., 2025) As a result, siRNA-mediated delivery elicits slower and weaker cytotoxic effects compared to delivery systems that induce more extensive endosomal damage.

Endosomal Escape Key Barriers and Open Questions

Endosomal escape is the critical bottleneck for modRNA-LNP therapeutics. Only 1-15% of internalized particles successfully release mRNA into the cytosol

Main Barriers

- **pH gradient**. Protonationof ionizable lipids destabilized the endosomal membrane, but the window is narrow (5-15 min)
- **Lipid geometry**. Branched or conical tails of the ionizable lipid promote curvature stress, but also raises toxicity
- **Particle size and number per cell**; too few results in low transfection, too many may lead to lysosomal stress and degradation
- **Cell type**: Hepatocytes and dendritic cells favour endosomal escape, quiescent or specialized cells like neurons or fibroblasts are less permissive

Unresolved Questions

- Is protein production driven by a few highly productive escape events, or many inefficient
- How do free ionizable lipids behave once released? (pKa shifts, ROS generation, immune activation, reactive aldehydes)?
- What happens to the modRNA immediately after escape (the 'dark hour of transfection") before translation begins?
- Do failed events contribute to chronic inflammation or lipid accumulation with repeated dosing
- How much variability is stochastic (intrinsic) vs cell type dependent and thus controllable?

Implication: Escape is both inefficient, unpredictable and context-dependent, leading to high variability in transfection and protein expression. Strategies to promote endosomal escape often increase cytotoxicity, resulting in the need for a better mechanistic understanding and safer lipid design

Single cell Analysis: a pharmacokinetic perspective

The pharmacokinetics of LNP delivery and protein expression are a complex, multi-step stochastic process involving uptake, endosomal processing, and mRNA escape. Using single-cell analysis, Müller *et al.* (Müller et al., 2024) found that cellular uptake was variable and ranged from minutes to hours depending on LNP shape, composition, and cell type. Endosomal escape varied among individual cells and was inversely related to protein production; faster release and translation of RNA led to increased protein output. A theoretical "area under the curve" (AUC), used to describe overall pharmaceutical protein availability, was found to depend equally on four factors: the number of mRNA molecules delivered, the translation rate, the mRNA lifetime, and the protein lifetime. Moreover, Müller *et al.* noted that little is known about the fate of nucleic acids after they escape from the endosome. Before any measurable action, such as protein expression occurs, there is what Müller calls "the dark hour of transfection," the intracellular biochemical and physical processes that occurs following endosomal escape but before protein synthesis. What happens during this period remains unclear, which limits a full understanding. Additionally, the amount of modRNA released into the cytosol does not reliably predict the level of protein expression, previously noted by Liu *et al.* (Liu et al., 2024)

2.7 Lipid Degradation and Metabolite Persistence

Once the modRNA is released, the fate of the lipid components determines the final pharmacodynamic stage of LNP activity. This aspect, concerning the fate of the individual lipids after they deliver their payload, is rarely discussed or addressed. The LNPs do not simply vanish; instead, they are disassembled *in vitro*, metabolized, and cleared at different rates depending on the lipid chemistry. For example, cholesterol may form oxysterols with immune effects, while DSPC can accumulate in organs, potentially altering membrane fluidity.

Both cholesterol and DSPC are natural lipids, but they are manufactured synthetically. Clearance pathways remain poorly characterized, necessitating further study. These are further delineated in **Table 4**.

PEGylated Lipids

The pegylated lipid plays a key role in the lipid matrix of the LNP, despite its small molar ratio, because it extends outward on its surface, which is necessary for LNP stability during formulation and storage.(Zhang et al., 2025) This also allows for increased *in vivo* circulation time since the PEG lipid impedes cellular uptake and endosomal escape, but this then creates the so-called "PEG dilemma." As a result, PEG-lipids with shorter C-14 acyl chains were used in the LNPs, which gradually diffused out of the particles and provided temporary stealth properties, achieving higher transfection efficiency than longer, more persistent PEG-lipids.(Mukai et al., 2022) Once the PEG-lipid is sloughed off, it is metabolized by the liver and kidneys, where the lipid component undergoes enzymatic hydrolysis and β-oxidation which is standard processes for lipids. The pegylated part, being a polymer of ethylene glycol, is either excreted in urine or broken down into smaller oligomers. Although PEG-lipids are designed to quickly detach from the LNP surface once in circulation or shortly after uptake, they can remain associated. Then they can be internalized with the particle and undergo endosomal trafficking to lysosomes, where the lipid portion is degraded and the PEG chains are either excreted or slowly metabolized.(Mui et al., 2013)

Ionizable Lipid

No clinical data exist for ALC-0315 and SM-102 regarding their retention and duration of activity in humans. Although they are labeled as "biodegradable" after their ester bonds are hydrolyzed within tissues and release their fatty acid tails, their overall ability to degrade doesn't truly improve, since common degradation pathways like β-oxidation are not consistently used. (Jörgensen et al., 2023)

Due to their sterically hindered ester structure, they are slowly hydrolyzed over several days. Jörgensen *et al.* highlight that these lipids usually have stable structures and multiple tertiary amines, which slow down their degradation and may cause toxicity. (Jörgensen et al., 2023) When ALC-0315 undergoes ester cleavage, it forms a doubly de-esterified metabolite that remains cationic and can reach metabolic sites such as mitochondrial membranes more quickly than longer lipids,(Eygeris et al., 2022; Jörgensen et al., 2023)possibly leading to ROS production, cytokine release and membrane disruption. As a result, the persistence of these shorter-chain lipids could lead to ongoing toxicity after exposure, (Hou et al., 2021; Inácio Â et al., 2011) but data in humans is sparse. Therefore, there is an urgent need to develop new combinatorial reactions that can generate degradable ionizable lipids for potent RNA delivery. (Han et al., 2021)

Lipid Adducts

An underrecognized risk for LNPs is the potential for lipid adduct formation, which occurs in storage. The head groups of tertiary amine-based lipids can form N-oxides and, consequently, fatty aldehyde impurities due to the thermodynamic instability of the LNPs and the oxidative impurities generated during the complex processing of the ionizable lipid.(Birdsall et al., 2024; Zhichang Yang, 2023) These aldehydes can react with modRNA nucleobases, especially adenine and cytidine, inside the LNP to form covalent bonds **(Figure 6e)**. Adduct levels increase with storage time and temperature, making the modRNA untranslatable once injected. Moderna scientists(Packer et al., 2021) first reported adduct formation in 2021, highlighting the lack of validated assays for detecting these adducts during manufacturing. Moderna also noted that the Tris buffer used in their product acts as an aldehyde sink,(Moderna, 2022) enabling more extended storage at 2–8°C and reducing adduct formation with the modRNA. Notably, Pfizer switched from PBS to Tris buffer in October 2021, raising questions about the amount and reactivity of adducts in their early batches (**Table 1**).

The damaged adducted modRNA, once taken up by the cell, may be perceived as abnormal or viral-like by cellular sensors, which may trigger inflammatory signals or interferon responses.(Cordes et al., 2025; Maelfait et al., 2020) Post-transcriptional interference, including adduct-induced damage, is hypothesized to contribute to systemic immune dysregulation, ribosomal stalling and collision with trailing ribosomes, and exaggerated inflammatory responses(Cordes et al., 2025), especially in vulnerable individuals.(Acevedo-Whitehouse & Bruno, 2023; Rigby & Rehwinkel, 2015) Research on secondary amines and reactive aldehydes (e.g., 4-HNE from lipid peroxidation) indicates they are cytotoxic and may affect protein folding or function, leading to the formation of neoantigens that can provoke undesired immune responses or contribute to oxidative stress and lysosomal dysfunction. (Bitounis et al., 2024; Dalleau et al., 2013; Fritz & Petersen, 2013) However, direct *in vivo* evidence of adduct formation after LNP uptake has not been confirmed. Moderna is actively exploring strategies to reduce covalent bonds and RNA-LNP adducts, acknowledging their potential toxicity.(Meredith Packer et al., 2022) Similarly, DNA-LNP adducts could form with residual DNA in the vaccines, potentially triggering interferon production.(Atianand & Fitzgerald, 2013) It is unclear whether BioNTech considers these phenomena. Alternative ionizable lipids with piperidine heads have been developed to mitigate this risk and enhance thermal stability.(Hashiba, Taguchi, Sakamoto, Otsu, Maeda, Ebe, et al., 2024) However, the risks of adduct formation anticipated by developers have not yet been systematically evaluated in vaccine studies.

The recent EMA draft guideline for modRNA vaccines (EuropeanMedicinesAgency, 2025) emphasizes the control of adduct formation in manufacturing but does not delineate the possible adverse effects. Continuous pharmacovigilance and advanced *in vivo* assays are essential to clarify these uncertainties *in vivo*, particularly for vulnerable groups. The lipid components, metabolic pathway, and knowledge gaps are summarized in **Table 4**.

.Lipid Component	Metabolic Pathway	Clearance	Persistence/Risks	Knowledge Gaps
Cholesterol	Sterol metabolism to HDL/LDL; possible oxidation to oxysterols	Likely recycled endogenously, but this has not been studied	Oxysterols are immunologically active; (Back et al., 2024) cholesterol crystals form depending on saturation, may contribute to CARPA (Anindita et al., 2024)	No direct oxysterol data available after LNP uptake,
DSPC (helper lipid)	Phospholipase degradation is incorporated into membranes. Displays unusual rigidity. (Li et al., 2015), favours bleb formation.(Simonsen, 2024; Zhang & Barz, 2025)	Days-weeks can accumulate in the liver, spleen, heart, kidney, lung (Quick et al., 2022)	DSPC can produce phospholipid-derived products that may alter membrane structure and stability (Jeschek et al., 2016); (Rezaei et al., 2025). Can affect lipid raft integrity and functions like increased T-cell signaling (Zech et al., 2009) May also lower immune host surveillance. (Sfera et al., 2022)	The effect on lipid rafts across tissues is not fully understood nor thoroughly examined with repeated dosing.
PEGylated lipids (ALC-0159, PEG-DMG) **See text**	Lipid moiety hydrolyzed, PEG excreted renally	Renal and hepatobiliary	PEG accumulation with repeated dosing; CARPA risk Vacuolations due to incomplete metabolism in lysosomes have been seen in animal studies (class effect) (TherapeuticGoodsAdministration, 2021) but not in humans(Obeng et al., 2025)	Human persistence and dose thresholds unclear; PEG allergy may limit LNP use for other indications(Song et al., 2025)
Ionizable lipids (ALC-0315, SM-102) **See text**	Hydrolysis to amines/fatty acids; branched tails resist β-oxidation (ALC-0315>>SM-102)	Slow hepatic clearance; ALC-0315 takes up to 3 weeks to fully metabolize ($t^{1}/_{2}$=139 hrs)(EMA/707383/, 2020 Corr.1*[1]) SM-102 half-life shorter at 7.3h(Y. Ren et al., 2025)	Tissue persistence of metabolites, including in mitochondria. In silico experiments demonstrate membrane embedding, which may enhance persistence(Aliakbarinodehi et al., 2024; Ermilova & Swenson, 2023) ROS production, cytokine release, membrane disruption or tearing	Identity of metabolites; long-term accumulation not well studied

Lipid Adducts **See text**	Reactive amines/aldehydes covalently bind proteins and nucleic acids(Packer et al., 2021)	Clearance uncertain	Persistent adducts, potential neoantigen formation, oxidative damage are possible	No standardized *in vivo* assays, frequency, and their impact *in vivo* are unknown.

735 **Table 4: Lipid Components, Metabolic Pathways and Knowledge Gaps** HDL=high density
736 lipoprotein; LDL=low density lipoprotein; PEG=pegylated lipid CARPA=complement activation
737 reaction pseudoallergy; ROS=reactive oxygen species

738

739 **2.8 Drug Interactions**

740

741 Although regulatory agencies generally assume vaccines do not cause drug–drug interactions, early evi-
742 dence suggests this may not hold for modRNA–LNP vaccines. Case reports and cohort analyses docu-
743 ment clinically relevant changes in clozapine pharmacokinetics post-vaccination, in some cases leading
744 to neutropenia and hospitalization (Bayraktar et al., 2021; Imai et al., 2022; Thompson et al., 2021). The
745 mechanism is consistent with inflammation-mediated suppression of CYP450 enzymes, particularly
746 CYP1A2 and CYP3A4, central to clozapine metabolism (Eiermann et al., 1997).

747

748 While most effects appear mild or transient (Demler & O'Donnell, 2023), therapeutic drug monitoring
749 has been recommended for narrow-index drugs like clozapine (Veerman et al., 2022). Substantial
750 increases in escitalopram, fluoxetine, trazodone, and quetiapine levels have also been reported (Kuzin
751 et al., 2023), and a case of neuroleptic malignant syndrome with adrenal insufficiency occurred in a
752 patient on valproic acid (Mizuno et al., 2022).

753

754 This concern extends beyond psychotropic or antiepileptic medications. Inflammatory cytokines such
755 as IL-6, TNF-α, and interferon-γ, induced by both infection and vaccination, down-regulate multiple
756 hepatic CYP isoenzymes (Lim et al., 2023). Clinical studies in COVID-19 patients have shown that
757 elevated C-reactive protein levels are associated with reduced metabolism of midazolam and tacrolimus,
758 potentially leading to oversedation or immunosuppressant toxicity.

759

760 Because many common drugs, such as statins, benzodiazepines, antiepileptics, and
761 immunosuppressants, are CYP3A4(Villemure et al., 2023) or CYP2C9 substrates(Lim et al., 2023),

762 transient suppression of these pathways after vaccination could alter drug exposure in a clinically
763 significant way. Yet regulators do not currently require pharmacokinetic interaction studies for
764 vaccines, leaving these risks under-characterized, and clinicians may be unaware.

765

766 The possible pharmacodynamic interactions with lipid nanoparticles themselves may be overlooked.
767 Recent work has shown that small-molecule drugs can directly influence endosomal trafficking and
768 escape. Tricyclic cationic amphiphilic drugs (TCADs), such as tricyclic antidepressants, first-generation
769 antipsychotics, and certain antihistamines, share structural features with ionizable lipids and have been
770 repurposed in experimental systems to improve intracellular delivery of nucleic acids.(Debisschop et al.,
771 2024) In animal studies, nortriptyline-containing "CADosomes" demonstrated delivery efficiency
772 without the need for synthetic ionizable lipids,(Bogaert et al., 2022) suggesting a structural and
773 functional overlap between cationic amphiliphic drugs (CADs) and LNP excipients. While this may be
774 exploited experimentally to enhance delivery, it raises the question of whether patients already taking
775 CAD-class drugs (e.g., antipsychotics, some antidepressants, etc) may experience altered LNP
776 trafficking or immune responses following vaccination.

777

Vaccine-Drug Interactions May Be Underreconized

Pharmacokinetic and pharmacodynamic interactions are not regularly evaluated during vaccine development, as regulatory agencies generally assume there are no clinically significant drug–vaccine interactions. (WorldHealthOrganization, 2005) However, rare case reports with influenza (Carnovale et al., 2018) and COVID-19 modRNA–LNP vaccines challenges this assumption.

Case reports of clozapine toxicity, (Thompson et al., 2021), observations of altered serum levels of antiepileptics,(Mizuno et al., 2022) and observational studies linking inflammation-induced cytokines to CYP450 suppression(F. Liu et al., 2023) all suggest a potential for transient but clinically significant interactions.

Additionally, lipid nanoparticles exhibit pharmacodynamic interactions, such as altered endosomal trafficking, which can occur in patients taking psychotropic or other medications and remain largely unexplored.

Overall, these gaps suggest that vaccine–drug interactions are both possible and clinically relevant, but are currently underestimated due to existing regulatory frameworks.

Other drug classes have also been implicated in modifying endosomal escape. Proton pump inhibitors, such as esomeprazole, have recently been investigated as adjuvants in preclinical LNP formulations, by raising endosomal pH, enhancing LNP delivery and immune responses via lysosomal destabilization in murine models.(Kim et al., 2025) PPI use has also been shown to increase risk of severe COVID-19 outcomes.(G.-F. Li et al., 2021) For chronic PPI users, altered transfection efficiency could amplify AEs, warranting caution and further research into vaccine safety profiles. These findings suggest that the LNP itself functions as a cationic amphiphilic drug (CAD), and its toxicological profile may overlap with that of CAD drugs (Gould & Templin, 2023). Endosomal escape enhancers, whether intentionally incorporated into formulations or present coincidentally in patient medications, can increase cytosolic release but also exacerbate lysosomal damage and galectin-mediated inflammation.(Dowdy, 2023; Omo-Lamai et al., 2025)This dual potential to both enhance efficacy and intensify toxicity underscores the need for pharmacovigilance analyses examining outcomes in patients on CADs, or other drugs at the time of vaccination. Together, these observations argue that vaccine–drug interactions are not only possible but clinically relevant, and their continued neglect in regulatory assessment represents a substantial oversight.

3 Challenges, Gaps and Future Directions

The modRNA-LNP platforms are transformative technologies with significant clinical potential. However, several critical uncertainties remain. These challenges come from the complex physicochemical properties of the technology and from broader translational and regulatory issues. As a result, there is an ongoing need for sustained mechanistic research and transparent long-term studies.

Category	Documented Challenges	Broader Uncertainties	Implications
Physicochemistry	Reliable characterization of particle size, encapsulation, payload, and stability remains challenging(Nogueira et al., 2024) No benchmark lipid formulation exists.(Simon et al., 2023) Standards and assays are continually evolving.(Pavlin et al., 2025; UnitedStatesPharmacopeia, 2024; Webb et al., 2025)	Black box formulation, the dynamic nature of LNPs results in unpredictable *in vitro* and *in vivo* behaviour	Comprehensive analytical standards are required, including proteomic and lipidomic profiling.
Biodistribution and Transfection	Conflation of biodistribution with gene expression,(Ci et al., 2023; Vervaeke et al., 2022) widespread off-target distribution.(Luo et al., 2025; Pateev et al., 2023)	Limited ability to achieve tissue-specific delivery beyond the liver.(Hosseini-Kharat M, 2025) Transfection is random and uneven; emerging tools like single-cell Nano mapping are still experimental.(Luo et al., 2025)	Therapeutic outcomes and adverse effects remain difficult to predict; single-cell methods are needed.
Protein Corona	Formation is dynamic, species-specific, and patient-dependent, affecting biodistribution and immune recognition. Levels of cell uptake do not correlate with increased mRNA translation likely due to protein corona-induced lysosomal trafficking (Voke et al., 2025) Measurement remains challenging.(Francia et al., 2024)	Patient variability (including age, sex, and comorbidities)(Sun et al., 2024) complicates predictability.	Results in nonlinear uptake, increased risk of immune activation, and reduced targeting accuracy.

Endosomal Escape	Low efficiency (1-15%); high stochastic cell-to-cell variability;(Johansson et al., 2025; Paramasivam et al., 2021) "dark hour" between escape and gene expression is not well understood.(Müller et al., 2024) Attempts to improve endosomal escape raise toxicity(Dowdy, 2023) LNPs alter cell membranes (Escalona-Rayo et al., 2024; Schlich et al., 2021)	Escape remains nonlinear, context-dependent, with a bottleneck that limits potency(Chatterjee et al., 2024; Johansson et al., 2025; Paramasivam et al., 2021)	"Bottleneck" increases unpredictability of therapeutic efficacy.(Chatterjee et al., 2024) Non-linear and context-dependent; bell-shaped curve(Bates et al., 2025)
Persistence and Lipid Metabolism	PEG-lipid immune effects,(Bakos et al., 2024), possible lysosomal stress,(Bitounis et al., 2024; Paramasivam et al., 2021), unknown toxic ionizable lipid metabolites,(Jörgensen et al., 2023), and cholesterol crystallization (Anindita et al., 2024), DSPC membrane effects	Long-term safety of repeated dosing remains unclear.	Risks of chronic accumulation, inflammation, or metabolic disruption may be possible; requires further investigation and focused studies
Manufacturing and stability	Documented batch heterogeneity;(EMA/707383/, 2020 Corr.1*[1]) instability in plasma; (Zhang & Barz, 2025) post-injection remodeling';(Y. Ren et al., 2025) cold-chain and scale-up challenges.(Oude Blenke et al., 2023) Lipid adducts an unrecognized concern	Effects of instability on potency and safety remain uncertain.	Variable potency, potential side effects, and administrative challenges can compromise efficacy and increase adverse event risk. Lipid adduct formation may affect therapeutic outcomes and AE profile
Drug Interactions	Case reports of clozapine toxicity(Thompson et al., 2021) and altered antiepileptic levels after vaccination;(Kow & Hasan, 2021) CYP450 suppression during inflammation is well established.(Villemure et al., 2023)	The degree to which modRNA–LNP vaccines transiently alter drug metabolism (CYP3A4, 2C9, 1A2) or interact with lysosomotropic drugs (e.g., psychotropics) or other drugs remains unknown	Vaccine–drug interactions are not systematically assessed; potential underrecognized risk for patients on narrow therapeutic index drugs (clozapine, tacrolimus, midazolam).

Regulatory and Data Gaps	LNPs have adjuvant-like activity, as acknowledged by the FDA,(Peden, 2022) but were classified as excipients in regulatory submissions. Pfizer/BioNTech's Comirnaty lacked transfection and target-cell-specific data, and CARPA was not assessed.(EMA/707383/, 2020 Corr.1*[1]) The FDA did not evaluate Moderna's LNPs separately.(Hemmrich & McNeil, 2023) New EMA guidelines on the quality of modRNA vaccines reinforce the classification of excipients.(EuropeanMedicinesAgency, 2025)	Current regulatory framework does not capture transfection and nanoparticle-specific risks; transparency and public trust remain unresolved issues. Drug interactions were not assessed	Incomplete safety evaluation, risks confusion, and skepticism. Advanced methods, including proteomics (Boros et al., 2024; EuropeanMedicinesAgency, 2025) and lipid profiling,(USFDA, 2022) are needed to fully characterize LNP–modRNA formulations and their pharmacological and immunostimulatory properties. Secondary pharmacology, drug interactions, assessment of long term risks required for regulatory assessment of LNPs

TABLE 5 Critical Uncertainties and Challenges of modRNA-LNP Technology

Considering the factors discussed, processing and administering into a living organism involves numerous disruptive factors. As a result, neither biodistribution nor transfection follows a linear pattern, and unpredictable variations in the measured values occur depending on the *in vivo* model.

It also remains plausible that both a nonlinear distribution pathway and the transfection rate, dependent on the formulation of the LNPs and the specific lipid components, may occur. From a pharmacokinetic perspective, the challenges associated with LNP technology, as identified in earlier research, have not been fully addressed.

These concerns are not isolated technical issues but interconnected challenges. The physicochemical heterogeneity and the dynamic structure of LNPs influence biodistribution, which in turn depends on the dynamic protein corona; meanwhile, inefficiencies in endosomal escape exacerbate variability in therapeutic outcomes. The toxicological dynamics of the extracellular LNPs are unstudied(Bitounis et al., 2024), as is the possibility of lysosomal stress or dysfunction which is increasingly linked to numerous diseases, such as neurodegenerative disorders.(Feng et al., 2024) Likewise, patient heterogeneity amplifies these uncertainties, making it unreasonable to expect uniform efficacy or safety across populations and making it difficult to predict clinical response or an adverse event profile. Gaps in regulatory requirements, such as critical quality attributes, target-cell specificity, biodistribution,(Vervaeke et al., 2022) immune effects, drug interactions, and long-term toxicology, further undermine public confidence and complicate post-marketing safety and surveillance.

We assert that the interplay between protein corona composition, cellular uptake pathways, endosomal escape and lipid metabolism critically influences cell tropism, protein production, and the stability of both the lipid and RNA components. These aspects should be carefully considered and require further investigation.

Given the dependencies shown, it is worth questioning whether parameters reliant on highly individual physiological factors, such as age-related metabolic changes, pre-existing conditions, medications, baseline protein levels, or temporal fluctuations in protein concentrations, can be effectively controlled or standardized.(J. Li et al., 2021; Wegler et al., 2019)

Furthermore, these factors are inherently difficult to quantify and measure because they vary on an individual basis, and because *in vitro* measurements do not always reflect the *in vivo* behaviour of this technology. This raises fundamental challenges for the translation of LNP-based therapeutics into clinical practice.

Discussion

Looking ahead, various strategies are being explored to address the unpredictability of current modRNA–LNP systems. One approach involves developing liposomal LNP hybrids, which may lower biocorona complexity and enable extra-hepatic targeting.(M. H. Y. Cheng et al., 2025) Exosome-inspired or engineered extracellular vesicles offer another promising avenue,(Iqbal et al., 2024) leveraging their natural ability to cross physiological barriers and evade immune clearance.(Maugeri et al., 2019)

On the chemistry front, new classes of ionizable lipids with improved degradation profiles are being developed to reduce persistence and toxicity.(Han et al., 2021; Jörgensen et al., 2023; Omo-Lamai et al., 2025) Simultaneously, advances in single-cell mapping technologies aim to clarify stochastic uptake and expression at unprecedented resolution,(Bates et al., 2025; Johansson et al., 2025; Luo et al., 2025; Müller et al., 2024) potentially making delivery more predictable. Improvements in assay methodology(Pavlin et al., 2025; Webb et al., 2025)and in formulations such as lyophilization(De & Ko, 2023) look promising. Together, these innovations and others suggest that although current formulations remain a biological "black box," an expanding toolkit is being developed to potentially make modRNA delivery more controllable, targeted, and safer.

These uncertainties highlight the nonlinear and context-dependent nature of LNP-modRNA interactions, suggesting a pathogen-like effect on the cell beyond its inherent cytotoxicity. Insights from cationic amphiphiles such as antipsychotic drugs may enhance the understanding of these complex particles.(Gould & Templin, 2023; Sfera et al., 2022)

Progress will likely require integrating advanced *in vitro* and *in vivo* models,(Bitounis et al., 2024) single-cell resolution technologies,(Luo et al., 2025) and standardized analytical frameworks(Simon et al., 2023; UnitedStatesPharmacopeia, 2024) to achieve this goal.

However, it must be considered that *in vitro* experiments with such a highly variable technology *in vivo* require a systems biology perspective. Neither membrane structural processes nor downstream signal transduction(Thiemicke & Neuert, 2023; Vijay & Gujral, 2020)follow linear dynamics.

Additionally, incorporating longitudinal human data and comprehensive regulatory strategies will be crucial to ensure both efficacy and long-term safety. This will be a challenging task given the nonlinear dynamic nature of this technology.(Fung et al., 2024)

Summary

To the best of our knowledge, this work is the first to systematically synthesize the current understanding of LNP properties while highlighting unresolved challenges that have become increasingly evident in recent years but remain insufficiently addressed in clinical applications.

Declaration of competing interest

The authors declare that they have no competing interests.

Author contribution

L.M. Gutschi and F. Seger wrote this manuscript equally and discussed every aspect. The manuscript was published with the consent of both authors. The authors used Grammarly to improve the manuscript's readability. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

Materials

All slides were created by F. Seger using BioRender (unless otherwise indicated) and are used under BioRender's non-commercial license.

Acknowledgements

Dr Susan Natsheh, MD for visuals and proofreading

References

Abbasi, R., Shineh, G., Mobaraki, M., Doughty, S., & Tayebi, L. (2023). Structural parameters of nanoparticles affecting their toxicity for biomedical applications: a review. *Journal of Nanoparticle Research*, *25*(3), 43. https://doi.org/10.1007/s11051-023-05690-w

Acevedo-Whitehouse, K., & Bruno, R. (2023). Potential health risks of mRNA-based vaccine therapy: A hypothesis. *Med Hypotheses*, *171*, 111015. https://doi.org/10.1016/j.mehy.2023.111015

Ait-Oudhia, S., Mager, D. E., & Straubinger, R. M. (2014). Application of Pharmacokinetic and Pharmacodynamic Analysis to the Development of Liposomal Formulations for Oncology. *Pharmaceutics*, *6*(1), 137-174. https://www.mdpi.com/1999-4923/6/1/137

Akhter, M. H., Khalilullah, H., Gupta, M., Alfaleh, M. A., Alhakamy, N. A., Riadi, Y., & Md, S. (2021). Impact of Protein Corona on the Biological Identity of Nanomedicine: Understanding the Fate of Nanomaterials in the Biological Milieu. *Biomedicines*, *9*(10). https://doi.org/10.3390/biomedicines9101496

Akinc, A., Maier, M. A., Manoharan, M., Fitzgerald, K., Jayaraman, M., Barros, S., Ansell, S., Du, X., Hope, M. J., Madden, T. D., Mui, B. L., Semple, S. C., Tam, Y. K., Ciufolini, M., Witzigmann, D., Kulkarni, J. A., van der Meel, R., & Cullis, P. R. (2019). The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. *Nature Nanotechnology*, *14*(12), 1084-1087. https://doi.org/10.1038/s41565-019-0591-y

Aliakbarinodehi, N., Niederkofler, S., Emilsson, G., Parkkila, P., Olsén, E., Jing, Y., Sjöberg, M., Agnarsson, B., Lindfors, L., & Höök, F. (2024). Time-Resolved Inspection of Ionizable Lipid-Facilitated Lipid Nanoparticle Disintegration and Cargo Release at an Early Endosomal Membrane Mimic. *ACS Nano*, *18*(34), 22989-23000. https://doi.org/10.1021/acsnano.4c04519

Aliyandi, A., Zuhorn, I. S., & Salvati, A. (2020). Disentangling Biomolecular Corona Interactions With Cell Receptors and Implications for Targeting of Nanomedicines. *Front Bioeng Biotechnol*, *8*, 599454. https://doi.org/10.3389/fbioe.2020.599454

Anindita, J., Tanaka, H., Yamakawa, T., Sato, Y., Matsumoto, C., Ishizaki, K., Oyama, T., Suzuki, S., Ueda, K., Higashi, K., Moribe, K., Sasaki, K., Ogura, Y., Yonemochi, E., Sakurai, Y., Hatakeyama, H., & Akita, H. (2024). The Effect of Cholesterol Content on the Adjuvant Activity of Nucleic-Acid-Free Lipid Nanoparticles. *Pharmaceutics*, *16*(2). https://doi.org/10.3390/pharmaceutics16020181

Atianand, M. K., & Fitzgerald, K. A. (2013). Molecular basis of DNA recognition in the immune system. *J Immunol*, *190*(5), 1911-1918. https://doi.org/10.4049/jimmunol.1203162

Back, P. I., Yu, M., Modaresahmadi, S., Hajimirzaei, S., Zhang, Q., Islam, M. R., Schwendeman, A. A., & La-Beck, N. M. (2024). Immune Implications of Cholesterol-Containing Lipid Nanoparticles. *ACS Nano*, *18*(42), 28480-28501. https://doi.org/10.1021/acsnano.4c06369

Baimanov, D., Wang, J., Liu, Y., Zheng, P., Yu, S., Liu, F., Wang, J., Boraschi, D., Zhao, Y., Chen, C., & Wang, L. (2025). Identification of Cell Receptors Responsible for Recognition and Binding of Lipid Nanoparticles. *Journal of the American Chemical Society*, *147*(9), 7604-7616. https://doi.org/10.1021/jacs.4c16987

Bakos, T., Mészáros, T., Kozma, G. T., Berényi, P., Facskó, R., Farkas, H., Dézsi, L., Heirman, C., de Koker, S., Schiffelers, R., Glatter, K. A., Radovits, T., Szénási, G., & Szebeni, J. (2024). mRNA-LNP COVID-19 Vaccine Lipids Induce Complement Activation and Production of Proinflammatory Cytokines: Mechanisms, Effects of Complement Inhibitors, and Relevance to Adverse Reactions. *Int J Mol Sci*, *25*(7). https://doi.org/10.3390/ijms25073595

Bates, S. M., Munson, M. J., Trovisco, V., Pereira, S., Miller, S. R., Sabirsh, A., Betts, C. J., Blenke, E. O., & Gay, N. J. (2025). The kinetics of endosomal disruption reveal differences in lipid nanoparticle induced cellular toxicity. *Journal of Controlled Release*, *386*, 114047. https://doi.org/https://doi.org/10.1016/j.jconrel.2025.114047

Bayraktar, İ., Yalçın, N., & Demirkan, K. (2021). The potential interaction between COVID-19 vaccines and clozapine: A novel approach for clinical trials. *Int J Clin Pract*, *75*(8), e14441. https://doi.org/10.1111/ijcp.14441

Behzadi, S., Serpooshan, V., Tao, W., Hamaly, M. A., Alkawareek, M. Y., Dreaden, E. C., Brown, D., Alkilany, A. M., Farokhzad, O. C., & Mahmoudi, M. (2017). Cellular uptake of nanoparticles: journey inside the cell. *Chem Soc Rev*, *46*(14), 4218-4244. https://doi.org/10.1039/c6cs00636a

Birdsall, R. E., Han, D., DeLaney, K., Kowalczyk, A., Cojocaru, R., Lauber, M., & Huray, J. L. (2024). Monitoring stability indicating impurities and aldehyde content in lipid nanoparticle raw material and formulated drugs. *Journal of Chromatography B*, *1234*, 124005. https://doi.org/https://doi.org/10.1016/j.jchromb.2024.124005

Bitounis, D., Jacquinet, E., Rogers, M. A., & Amiji, M. M. (2024). Strategies to reduce the risks of mRNA drug and vaccine toxicity. *Nature Reviews Drug Discovery*, *23*(4), 281-300. https://doi.org/10.1038/s41573-023-00859-3

Bogaert, B., Sauvage, F., Guagliardo, R., Muntean, C., Nguyen, V. P., Pottie, E., Wels, M., Minnaert, A.-K., De Rycke, R., Yang, Q., Peer, D., Sanders, N., Remaut, K., Paulus, Y. M., Stove, C., De Smedt, S. C., & Raemdonck, K. (2022). A lipid nanoparticle platform for mRNA delivery through repurposing of cationic amphiphilic drugs. *Journal of Controlled Release*, *350*, 256-270. https://doi.org/https://doi.org/10.1016/j.jconrel.2022.08.009

Borah, A., Giacobbo, V., Binici, B., Baillie, R., & Perrie, Y. (2025). From in vitro to in vivo: The Dominant role of PEG-Lipids in LNP performance. *European Journal of Pharmaceutics and Biopharmaceutics*, *212*, 114726. https://doi.org/https://doi.org/10.1016/j.ejpb.2025.114726

Boros, L. G., Kyriakopoulos, A. M., Brogna, C., Piscopo, M., McCullough, P. A., & Seneff, S. (2024). Long-lasting, biochemically modified mRNA, and its frameshifted recombinant spike proteins in human tissues and circulation after COVID-19 vaccination. *Pharmacology Research & Perspectives*, *12*(3), e1218. https://doi.org/https://doi.org/10.1002/prp2.1218

Bost, J. P., Barriga, H., Holme, M. N., Gallud, A., Maugeri, M., Gupta, D., Lehto, T., Valadi, H., Esbjörner, E. K., Stevens, M. M., & El-Andaloussi, S. (2021). Delivery of Oligonucleotide Therapeutics: Chemical Modifications, Lipid Nanoparticles, and Extracellular Vesicles. *ACS Nano*, *15*(9), 13993-14021. https://doi.org/10.1021/acsnano.1c05099

Burdette, D., Ci, L., Shilliday, B., Slauter, R., Auerbach, A., Kenney, M., Almarsson, Ö., Cheung, E., & Hendrick, T. (2023). Systemic Exposure, Metabolism, and Elimination of [¹⁴C]-Labeled Amino Lipid, Lipid 5, after a Single Administration of mRNA Encapsulating Lipid Nanoparticles to Sprague-Dawley Rats. *Drug Metabolism and Disposition*, *51*(7), 804-812. https://doi.org/10.1124/dmd.122.001194

Carnovale, C., Raschi, E., Leonardi, L., Moretti, U., De Ponti, F., Gentili, M., Pozzi, M., Clementi, E., Poluzzi, E., & Radice, S. (2018). No signal of interactions between influenza vaccines and drugs used for chronic diseases: a case-by-case analysis of the vaccine adverse event reporting system and vigibase. *Expert Rev Vaccines*, *17*(4), 363-381. https://doi.org/10.1080/14760584.2018.1442718

Cedervall, T., Lynch, I., Lindman, S., Berggård, T., Thulin, E., Nilsson, H., Dawson, K. A., & Linse, S. (2007). Understanding the nanoparticle–protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *Proceedings of the National Academy of Sciences*, *104*(7), 2050-2055. https://doi.org/doi:10.1073/pnas.0608582104

CentresforDiseaseControlandPrevention. (2022). *Understanding mRNA COVID-19 Vaccines*. Retrieved 26 September 2025 from https://web.archive.org/web/20220722133644/https:/www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/mrna.html

Chatterjee, S., Kon, E., Sharma, P., & Peer, D. (2024). Endosomal escape: A bottleneck for LNP-mediated therapeutics. *Proceedings of the National Academy of Sciences*, *121*(11), e2307800120. https://doi.org/doi:10.1073/pnas.2307800120

Chaudhary, N., Kasiewicz, L. N., Newby, A. N., Arral, M. L., Yerneni, S. S., Melamed, J. R., LoPresti, S. T., Fein, K. C., Strelkova Petersen, D. M., Kumar, S., Purwar, R., & Whitehead, K. A. (2024). Amine headgroups in ionizable lipids drive immune responses to lipid nanoparticles by binding to the receptors TLR4 and CD1d. *Nature Biomedical Engineering*, *8*(11), 1483-1498. https://doi.org/10.1038/s41551-024-01256-w

Chen, C., Chen, C., Li, Y., Gu, R., & Yan, X. (2023). Characterization of lipid-based nanomedicines at the single-particle level. *Fundamental Research*, *3*(4), 488-504. https://doi.org/https://doi.org/10.1016/j.fmre.2022.09.011

Chen, J., Xu, Y., Zhou, M., Xu, S., Varley, A. J., Golubovic, A., Lu, R. X. Z., Wang, K. C., Yeganeh, M., Vosoughi, D., & Li, B. (2023). Combinatorial design of ionizable lipid nanoparticles for muscle-selective mRNA delivery with minimized off-target effects. *Proc Natl Acad Sci U S A*, *120*(50), e2309472120. https://doi.org/10.1073/pnas.2309472120

Chen, X., Ye, Y., Li, M., Zuo, T., Xie, Z., Ke, Y., Cheng, H., Hong, L., & Liu, Z. (2025). Structural characterization of mRNA lipid nanoparticles (LNPs) in the presence of mRNA-free LNPs. *Journal of Controlled Release*, *386*, 114082. https://doi.org/https://doi.org/10.1016/j.jconrel.2025.114082

Cheng, M. H. Y., Zhang, Y., Fox, K., Leung, J., Strong, C., Kang, E., Chen, Y., Tong, M., Bommadevara, H., Jan, E., Ip, O. Y. L., Rodríguez-Rodríguez, C., Saatchi, K., Häfeli, U. O., Abdolahzadeh, A., Witzigmann, D., & Cullis, P. R. (2025). Liposomal lipid nanoparticles for extrahepatic delivery of mRNA. *Nature Communications*, *16*(1), 4135. https://doi.org/10.1038/s41467-025-58523-w

Cheng, Y., Zhao, E., Yang, X., Luo, C., Zi, G., Wang, R., Xu, Y., & Peng, B. (2025). Entrapment of lipid nanoparticles in peripheral endosomes but not lysosomes impairs intracellular trafficking and endosomal escape. *International Journal of Pharmaceutics*, *669*, 125024. https://doi.org/https://doi.org/10.1016/j.ijpharm.2024.125024

Ci, L., Hard, M., Zhang, H., Gandham, S., Hua, S., Wickwire, J., Wehrman, T., Slauter, R., Auerbach, A., Kenney, M., Mercer, G., Hendrick, T., Almarsson, Ö., Cheung, E., & Burdette, D. (2023). Biodistribution of Lipid 5, mRNA, and Its Translated Protein Following Intravenous Administration of mRNA-Encapsulated Lipid Nanoparticles in Rats. *Drug Metab Dispos*, *51*(7), 813-823. https://doi.org/10.1124/dmd.122.000980

Cordes, J., Zhao, S., Engel, C. M., & Stingele, J. (2025). Cellular responses to RNA damage. *Cell*, *188*(4), 885-900. https://doi.org/https://doi.org/10.1016/j.cell.2025.01.005

Cullis, P. R., & Felgner, P. L. (2024). The 60-year evolution of lipid nanoparticles for nucleic acid delivery. *Nature Reviews Drug Discovery*, *23*(9), 709-722. https://doi.org/10.1038/s41573-024-00977-6

da Costa Marques, R., Hüppe, N., Speth, K. R., Oberländer, J., Lieberwirth, I., Landfester, K., & Mailänder, V. (2023). Proteomics reveals time-dependent protein corona changes in the intracellular pathway. *Acta Biomaterialia*, *172*, 355-368. https://doi.org/https://doi.org/10.1016/j.actbio.2023.10.010

Dalleau, S., Baradat, M., Guéraud, F., & Huc, L. (2013). Cell death and diseases related to oxidative stress:4-hydroxynonenal (HNE) in the balance. *Cell Death & Differentiation*, *20*(12), 1615-1630. https://doi.org/10.1038/cdd.2013.138

De, A., & Ko, Y. T. (2023). Why mRNA-ionizable LNPs formulations are so short-lived: causes and way-out. *Expert Opinion on Drug Delivery*, *20*(2), 175-187. https://doi.org/10.1080/17425247.2023.2162876

De, C. K., Tsuda, M., Zhu, C., Dehn, S., Hinrichs, H., Tsuji, N., Jin, H., Arase, H., Tanaka, S., & List, B. (2025). The Overlooked Stereoisomers of the Ionizable Lipid ALC315. *Journal of the American Chemical Society*. https://doi.org/10.1021/jacs.5c08345

Debisschop, A., Bogaert, B., Muntean, C., De Smedt, S. C., & Raemdonck, K. (2024). Beyond chloroquine: Cationic amphiphilic drugs as endosomal escape enhancers for nucleic acid therapeutics. *Current Opinion in Chemical Biology*, *83*, 102531. https://doi.org/https://doi.org/10.1016/j.cbpa.2024.102531

Demler, T. L., & O'Donnell, C. (2023). Exploring the Impact of COVID-19 Vaccination on Patients Taking Clozapine. *Innov Clin Neurosci*, *20*(1-3), 32-38.

Dey, A. K., Nougarède, A., Clément, F., Fournier, C., Jouvin-Marche, E., Escudé, M., Jary, D., Navarro, F. P., & Marche, P. N. (2021). Tuning the Immunostimulation Properties of Cationic Lipid Nanocarriers for Nucleic Acid Delivery [Original Research]. *Frontiers in Immunology*, *Volume 12 - 2021*. https://doi.org/10.3389/fimmu.2021.722411

Di, J., Du, Z., Wu, K., Jin, S., Wang, X., Li, T., & Xu, Y. (2022). Biodistribution and Non-linear Gene Expression of mRNA LNPs Affected by Delivery Route and Particle Size. *Pharm Res*, *39*(1), 105-114. https://doi.org/10.1007/s11095-022-03166-5

Dowdy, S. F. (2023). Endosomal escape of RNA therapeutics: How do we solve this rate-limiting problem? *Rna*, *29*(4), 396-401. https://doi.org/10.1261/rna.079507.122

Dowdy, S. F., Setten, R. L., Cui, X. S., & Jadhav, S. G. (2022). Delivery of RNA Therapeutics: The Great Endosomal Escape! *Nucleic Acid Ther*, *32*(5), 361-368. https://doi.org/10.1089/nat.2022.0004

Eiermann, B., Engel, G., Johansson, I., Zanger, U. M., & Bertilsson, L. (1997). The involvement of CYP1A2 and CYP3A4 in the metabolism of clozapine. *British journal of clinical pharmacology*, *44*(5), 439-446. https://doi.org/10.1046/j.1365-2125.1997.t01-1-00605.x

EMA/707383/. (2020 Corr.1*[1]). *Comirnaty European Public Assessment Report (EPAR)*. https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf: European Medicines Agency Retrieved from https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf

EMEA/H/C/005735/RR. (2020). *Rappaporteur's Rolling Review Report Overview LoQ-COVID-19 mRNA Vaccine BioNTech*. https://www.covidtruths.co.uk/2021/04/ema-leaked-papers/: European Medicines Agency Retrieved from https://www.covidtruths.co.uk/2021/04/ema-leaked-papers/

Er-Rafik, M., Ferji, K., Combet, J., Sandre, O., Lecommandoux, S., Schmutz, M., Le Meins, J.-F., & Marques, C. M. (2022). Tear of lipid membranes by nanoparticles [10.1039/D2SM00179A]. *Soft Matter*, *18*(17), 3318-3322. https://doi.org/10.1039/D2SM00179A

Ermilova, I., & Swenson, J. (2023). Ionizable lipids penetrate phospholipid bilayers with high phase transition temperatures: perspectives from free energy calculations. *Chemistry and Physics of Lipids*, *253*, 105294. https://doi.org/https://doi.org/10.1016/j.chemphyslip.2023.105294

Escalona-Rayo, O., Papadopoulou, P., Slütter, B., & Kros, A. (2024). Biological recognition and cellular trafficking of targeted RNA-lipid nanoparticles. *Current Opinion in Biotechnology*, *85*, 103041. https://doi.org/https://doi.org/10.1016/j.copbio.2023.103041

EuopeanMedicinesAgency. (2021). *Covid-19 Vaccine Moderna European Public Assessment Report (EPAR)*. https://www.ema.europa.eu/en/documents/assessment-report/spikevax-previously-covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf: EMA Retrieved from https://www.ema.europa.eu/en/documents/assessment-report/spikevax-previously-covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf

EuropeanMedicinesAgency. (2025). *Guidelines on the quality aspects of mRNA vaccines: draft* www,ema.europe.eu/en/documents/scientific-guideline/drat-guideline-quality-aspects-mrna-vaccines_en.pdf: EuropeanMedicines Agency

Eygeris, Y., Gupta, M., Kim, J., & Sahay, G. (2022). Chemistry of Lipid Nanoparticles for RNA Delivery. *Acc Chem Res*, *55*(1), 2-12. https://doi.org/10.1021/acs.accounts.1c00544

Fell, V. H. K., Kramer, T., Heindl, A., & Merkel, O. M. (2025). Prediction of the Apparent pKa Value of Lipid Nanoparticles by Density Functional Theory. *ACS Materials Au*, *5*(3), 451-457. https://doi.org/10.1021/acsmaterialsau.4c00158

Feng, Y., Fu, H., Zhang, X., Liu, S., & Wei, X. (2024). Lysosome toxicities induced by nanoparticle exposure and related mechanisms. *Ecotoxicology and Environmental Safety*, *286*, 117215. https://doi.org/https://doi.org/10.1016/j.ecoenv.2024.117215

Francia, V., Zhang, Y., Cheng, M. H. Y., Schiffelers, R. M., Witzigmann, D., & Cullis, P. R. (2024). A magnetic separation method for isolating and characterizing the biomolecular corona of lipid nanoparticles. *Proc Natl Acad Sci U S A*, *121*(11), e2307803120. https://doi.org/10.1073/pnas.2307803120

Fritz, K. S., & Petersen, D. R. (2013). An overview of the chemistry and biology of reactive aldehydes. *Free Radical Biology and Medicine*, *59*, 85-91. https://doi.org/https://doi.org/10.1016/j.freeradbiomed.2012.06.025

Fung, S. Y. S., Xǔ, X. J., & Wu, M. (2024). Nonlinear dynamics in phosphoinositide metabolism. *Current Opinion in Cell Biology*, *88*, 102373. https://doi.org/https://doi.org/10.1016/j.ceb.2024.102373

Gould, S., & Templin, M. V. (2023). Off target toxicities and links with physicochemical properties of medicinal products, including antibiotics, oligonucleotides, lipid nanoparticles (with cationic and/or anionic charges). Data review suggests an emerging pattern. *Toxicol Lett*, *384*, 14-29. https://doi.org/10.1016/j.toxlet.2023.07.011

Haghighi, E., Abolmaali, S. S., Dehshahri, A., Mousavi Shaegh, S. A., Azarpira, N., & Tamaddon, A. M. (2024). Navigating the intricate in-vivo journey of lipid nanoparticles tailored for the targeted delivery of RNA therapeutics: a quality-by-design approach. *J Nanobiotechnology*, *22*(1), 710. https://doi.org/10.1186/s12951-024-02972-w

Hald Albertsen, C., Kulkarni, J. A., Witzigmann, D., Lind, M., Petersson, K., & Simonsen, J. B. (2022). The role of lipid components in lipid nanoparticles for vaccines and gene therapy. *Adv Drug Deliv Rev*, *188*, 114416. https://doi.org/10.1016/j.addr.2022.114416

Han, X., Zhang, H., Butowska, K., Swingle, K. L., Alameh, M.-G., Weissman, D., & Mitchell, M. J. (2021). An ionizable lipid toolbox for RNA delivery. *Nature Communications*, *12*(1), 7233. https://doi.org/10.1038/s41467-021-27493-0

Hashiba, K., Taguchi, M., Sakamoto, S., Otsu, A., Maeda, Y., Ebe, H., Okazaki, A., Harashima, H., & Sato, Y. (2024). Overcoming thermostability challenges in mRNA–lipid nanoparticle systems with piperidine-based ionizable lipids. *Communications Biology*, *7*(1), 556. https://doi.org/10.1038/s42003-024-06235-0

Hashiba, K., Taguchi, M., Sakamoto, S., Otsu, A., Maeda, Y., Suzuki, Y., Ebe, H., Okazaki, A., Harashima, H., & Sato, Y. (2024). Impact of Lipid Tail Length on the Organ Selectivity of mRNA-Lipid Nanoparticles. *Nano Letters*, *24*(41), 12758-12767. https://doi.org/10.1021/acs.nanolett.4c02566

Hassett, K. J., Rajlic, I. L., Bahl, K., White, R., Cowens, K., Jacquinet, E., & Burke, K. E. (2024). mRNA vaccine trafficking and resulting protein expression after intramuscular administration. *Molecular Therapy - Nucleic Acids*, *35*(1). https://doi.org/10.1016/j.omtn.2023.102083

He, H., Yuan, D., Wu, Y., & Cao, Y. (2019). Pharmacokinetics and Pharmacodynamics Modeling and Simulation Systems to Support the Development and Regulation of Liposomal Drugs. *Pharmaceutics*, *11*(3), 110. https://www.mdpi.com/1999-4923/11/3/110

He, Y., Wang, Y., Wang, L., Jiang, W., & Wilhelm, S. (2024). Understanding nanoparticle-liver interactions in nanomedicine. *Expert Opin Drug Deliv*, *21*(6), 829-843. https://doi.org/10.1080/17425247.2024.2375400

Hemmrich, E., & McNeil, S. (2023). Active ingredient vs excipient debate for nanomedicines. *Nature Nanotechnology*. https://doi.org/10.1038/s41565-023-01371-w

Hermosilla, J., Alonso-García, A., Salmerón-García, A., Cabeza-Barrera, J., Medina-Castillo, A. L., Pérez-Robles, R., & Navas, N. (2023). Analysing the In-Use Stability of mRNA-LNP COVID-

19 Vaccines Comirnaty™ (Pfizer) and Spikevax™ (Moderna): A Comparative Study of the Particulate. *Vaccines*, *11*(11), 1635. https://www.mdpi.com/2076-393X/11/11/1635

Hosseini-Kharat M, B. K., Prestige CA. (2025). Why do lipid nanoparticles target the liver? Understanding of biodistribution and liver-specific tropism [Review]. *Molecular Therapy Methods and Clinical Development*, *Volume 33*(1). https://www.cell.com/molecular-therapy-family/methods/fulltext/S2329-0501(25)00031-2

Hou, X., Zaks, T., Langer, R., & Dong, Y. (2021). Lipid nanoparticles for mRNA delivery. *Nature Reviews Materials*, *6*(12), 1078-1094. https://doi.org/10.1038/s41578-021-00358-0

Huang, T., Wang, J., Stukalov, A., Donovan, M. K. R., Ferdosi, S., Williamson, L., Just, S., Castro, G., Cantrell, L. S., Elgierari, E., Benz, R. W., Huang, Y., Motamedchaboki, K., Hakimi, A., Arrey, T., Damoc, E., Kreimer, S., Farokhzad, O. C., Batzoglou, S., . . . Hornburg, D. (2023). Protein Coronas on Functionalized Nanoparticles Enable Quantitative and Precise Large-Scale Deep Plasma Proteomics. *bioRxiv*, 2023.2008.2028.555225. https://doi.org/10.1101/2023.08.28.555225

Imai, T., Ochiai, S., Ishimaru, T., Daitoku, H., Miyagawa, Y., Fukuhara, R., Boku, S., & Takebayashi, M. (2022). A case report: Clozapine-induced leukopenia and neutropenia after mRNA COVID-19 vaccination. *Neuropsychopharmacol Rep*, *42*(2), 238-240. https://doi.org/10.1002/npr2.12238

Inácio Â, S., Mesquita, K. A., Baptista, M., Ramalho-Santos, J., Vaz, W. L., & Vieira, O. V. (2011). In vitro surfactant structure-toxicity relationships: implications for surfactant use in sexually transmitted infection prophylaxis and contraception. *PLOS ONE*, *6*(5), e19850. https://doi.org/10.1371/journal.pone.0019850

Iqbal, Z., Rehman, K., Mahmood, A., Shabbir, M., Liang, Y., Duan, L., & Zeng, H. (2024). Exosome for mRNA delivery: strategies and therapeutic applications. *J Nanobiotechnology*, *22*(1), 395. https://doi.org/10.1186/s12951-024-02634-x

Jeschek, D., Lhota, G., Wallner, J., & Vorauer-Uhl, K. (2016). A versatile, quantitative analytical method for pharmaceutical relevant lipids in drug delivery systems. *Journal of Pharmaceutical and Biomedical Analysis*, *119*, 37-44. https://doi.org/https://doi.org/10.1016/j.jpba.2015.11.020

Ji, Q., Zhu, H., Qin, Y., Zhang, R., Wang, L., Zhang, E., Zhou, X., & Meng, R. (2024). GP60 and SPARC as albumin receptors: key targeted sites for the delivery of antitumor drugs. *Frontiers in pharmacology*, *15*, 1329636. https://doi.org/10.3389/fphar.2024.1329636

Johansson, J. M., Du Rietz, H., Hedlund, H., Eriksson, H. C., Oude Blenke, E., Pote, A., Harun, S., Nordenfelt, P., Lindfors, L., & Wittrup, A. (2025). Cellular and biophysical barriers to lipid nanoparticle mediated delivery of RNA to the cytosol. *Nature Communications*, *16*(1), 5354. https://doi.org/10.1038/s41467-025-60959-z

Jörgensen, A. M., Wibel, R., & Bernkop-Schnürch, A. (2023). Biodegradable Cationic and Ionizable Cationic Lipids: A Roadmap for Safer Pharmaceutical Excipients. *Small*, *19*(17), 2206968. https://doi.org/https://doi.org/10.1002/smll.202206968

Kent, S. J., Li, S., Amarasena, T. H., Reynaldi, A., Lee, W. S., Leeming, M. G., O'Connor, D. H., Nguyen, J., Kent, H. E., Caruso, F., Juno, J. A., Wheatley, A. K., Davenport, M. P., & Ju, Y. (2024). Blood Distribution of SARS-CoV-2 Lipid Nanoparticle mRNA Vaccine in Humans. *ACS Nano*, *18*(39), 27077-27089. https://doi.org/10.1021/acsnano.4c11652

Khare, P., Edgecomb, S. X., Hamadani, C. M., Tanner, E. E. L., & S Manickam, D. (2023). Lipid nanoparticle-mediated drug delivery to the brain. *Advanced Drug Delivery Reviews*, *197*, 114861. https://doi.org/https://doi.org/10.1016/j.addr.2023.114861

Kim, H. J., Kim, H., Lee, J. H., & Hwangbo, C. (2023). Toll-like receptor 4 (TLR4): new insight immune and aging. *Immun Ageing*, *20*(1), 67. https://doi.org/10.1186/s12979-023-00383-3

Kim, Y. A., Jeong, H., Kim, H., Lee, S., Kim, K. S., & Na, K. (2025). Lipid nanoparticles with prazole adjuvant to enhance the efficacy of mRNA cancer vaccines. *Journal of Controlled Release*, *383*, 113756. https://doi.org/https://doi.org/10.1016/j.jconrel.2025.113756

Kloczewiak, M., Banks, J. M., Jin, L., & Brader, M. L. (2022). A Biopharmaceutical Perspective on Higher-Order Structure and Thermal Stability of mRNA Vaccines. *Molecular Pharmaceutics*, *19*(7), 2022-2031. https://doi.org/10.1021/acs.molpharmaceut.2c00092

Korzun, T., Moses, A. S., Diba, P., Sattler, A. L., Taratula, O. R., Sahay, G., Taratula, O., & Marks, D. L. (2023). From Bench to Bedside: Implications of Lipid Nanoparticle Carrier Reactogenicity for Advancing Nucleic Acid Therapeutics. *Pharmaceuticals*, *16*(8), 1088. https://www.mdpi.com/1424-8247/16/8/1088

Kow, C. S., & Hasan, S. S. (2021). Potential interactions between COVID-19 vaccines and antiepileptic drugs. *Seizure*, *86*, 80-81. https://doi.org/10.1016/j.seizure.2021.01.021

Kuzin, M., Gardin, F., Götschi, M., Xepapadakos, F., Kawohl, W., Seifritz, E., Trauzeddel, A., Paulzen, M., & Schoretsanitis, G. (2023). Changes in Psychotropic Drug Blood Levels After SARS-CoV-2 Vaccination: A Two-Center Cohort Study. *Therapeutic Drug Monitoring*, *45*(6), 792-796. https://doi.org/10.1097/ftd.0000000000001118

Lavington, S., & Watts, A. (2020). Lipid nanoparticle technologies for the study of G protein-coupled receptors in lipid environments. *Biophys Rev*, *12*(6), 1287-1302. https://doi.org/10.1007/s12551-020-00775-5

Lee, Y., Jeong, M., Park, J., Jung, H., & Lee, H. (2023). Immunogenicity of lipid nanoparticles and its impact on the efficacy of mRNA vaccines and therapeutics. *Experimental & Molecular Medicine*, *55*(10), 2085-2096. https://doi.org/10.1038/s12276-023-01086-x

Li, G.-F., An, X.-X., Yu, Y., Jiao, L.-R., Canarutto, D., Yu, G., Wang, G., Wu, D.-N., & Xiao, Y. (2021). Do proton pump inhibitors influence SARS-CoV-2 related outcomes? A meta-analysis. *Gut*, *70*(9), 1806-1808. https://doi.org/10.1136/gutjnl-2020-323366

Li, J., Cai, Z., Vaites, L. P., Shen, N., Mitchell, D. C., Huttlin, E. L., Paulo, J. A., Harry, B. L., & Gygi, S. P. (2021). Proteome-wide mapping of short-lived proteins in human cells. *Molecular Cell*, *81*(22), 4722-4735.e4725. https://doi.org/https://doi.org/10.1016/j.molcel.2021.09.015

Li, J., Wang, X., Zhang, T., Wang, C., Huang, Z., Luo, X., & Deng, Y. (2015). A review on phospholipids and their main applications in drug delivery systems. *Asian Journal of Pharmaceutical Sciences*, *10*(2), 81-98. https://doi.org/https://doi.org/10.1016/j.ajps.2014.09.004

Li, S., Hu, Y., Li, A., Lin, J., Hsieh, K., Schneiderman, Z., Zhang, P., Zhu, Y., Qiu, C., Kokkoli, E., Wang, T. H., & Mao, H. Q. (2022). Payload distribution and capacity of mRNA lipid nanoparticles. *Nat Commun*, *13*(1), 5561. https://doi.org/10.1038/s41467-022-33157-4

Liao, S., Wang, S., Wadhwa, A., Birkenshaw, A., Fox, K., Cheng, M. H. Y., Casmil, I. C., Magana, A. A., Bathula, N. V., Ho, C. H., Cheng, J.-Y., Foster, L. J., Harder, K. W., Ross, C. J. D., Cullis, P. R., & Blakney, A. K. (2025). Transfection Potency of Lipid Nanoparticles Containing mRNA Depends on Relative Loading Levels. *ACS Applied Materials & Interfaces*, *17*(2), 3097-3105. https://doi.org/10.1021/acsami.4c20077

Liau, B., Zhang, L., Ang, M. J. Y., Ng, J. Y., C.V, S. B., Schneider, S., Gudihal, R., Bae, K. H., & Yang, Y. Y. (2024). Quantitative analysis of mRNA-lipid nanoparticle stability in human plasma and serum by size-exclusion chromatography coupled with dual-angle light scattering. *Nanomedicine: Nanotechnology, Biology and Medicine*, *58*, 102745. https://doi.org/https://doi.org/10.1016/j.nano.2024.102745

Lim, S. Y. M., Al Bishtawi, B., & Lim, W. (2023). Role of Cytochrome P450 2C9 in COVID-19 Treatment: Current Status and Future Directions. *European Journal of Drug Metabolism and Pharmacokinetics*, *48*(3), 221-240. https://doi.org/10.1007/s13318-023-00826-8

Liu, F., Aulin, L. B. S., Manson, M. L., Krekels, E. H. J., & van Hasselt, J. G. C. (2023). Unraveling the Effects of Acute Inflammation on Pharmacokinetics: A Model-Based Analysis Focusing on Renal Glomerular Filtration Rate and Cytochrome P450 3A4-Mediated Metabolism. *Eur J Drug Metab Pharmacokinet*, *48*(6), 623-631. https://doi.org/10.1007/s13318-023-00852-6

Liu, F., & Hutchinson, R. (2024). Visible particles in parenteral drug products: A review of current safety assessment practice. *Current Research in Toxicology*, *7*, 100175. https://doi.org/https://doi.org/10.1016/j.crtox.2024.100175

Liu, H., Chen, M. Z., Payne, T., Porter, C. J. H., Pouton, C. W., & Johnston, A. P. R. (2024). Beyond the Endosomal Bottleneck: Understanding the Efficiency of mRNA/LNP Delivery. *Advanced Functional Materials*, *34*(39), 2404510. https://doi.org/https://doi.org/10.1002/adfm.202404510

Liu, K., Nilsson, R., Lázaro-Ibáñez, E., Duàn, H., Miliotis, T., Strimfors, M., Lerche, M., Salgado Ribeiro, A. R., Ulander, J., Lindén, D., Salvati, A., & Sabirsh, A. (2023). Multiomics analysis of

naturally efficacious lipid nanoparticle coronas reveals high-density lipoprotein is necessary for their function. *Nature Communications*, *14*(1), 4007. https://doi.org/10.1038/s41467-023-39768-9

Lonez, C., Vandenbranden, M., & Ruysschaert, J.-M. (2008). Cationic liposomal lipids: From gene carriers to cell signaling. *Progress in Lipid Research*, *47*(5), 340-347. https://doi.org/https://doi.org/10.1016/j.plipres.2008.03.002

Lonez, C., Vandenbranden, M., & Ruysschaert, J.-M. (2012). Cationic lipids activate intracellular signaling pathways. *Advanced Drug Delivery Reviews*, *64*(15), 1749-1758. https://doi.org/https://doi.org/10.1016/j.addr.2012.05.009

Luo, J., Molbay, M., Chen, Y., Horvath, I., Kadletz, K., Kick, B., Zhao, S., Al-Maskari, R., Singh, I., Ali, M., Bhatia, H. S., Minde, D.-P., Negwer, M., Hoeher, L., Calandra, G. M., Groschup, B., Su, J., Kimna, C., Rong, Z., . . . Erturk, A. (2025). Nanocarrier imaging at single-cell resolution across entire mouse bodies with deep learning. *Nature Biotechnology*. https://doi.org/10.1038/s41587-024-02528-1

Maelfait, J., Liverpool, L., & Rehwinkel, J. (2020). Nucleic Acid Sensors and Programmed Cell Death. *J Mol Biol*, *432*(2), 552-568. https://doi.org/10.1016/j.jmb.2019.11.016

Martins, A. M., Palomba, R., Schlich, M., & Decuzzi, P. (2024). On the axonal transport of lipid nanoparticles in primary hippocampal neurons. *Journal of Drug Delivery Science and Technology*, *101*, 106282. https://doi.org/https://doi.org/10.1016/j.jddst.2024.106282

Matthessen, R., Van Pottelberge, R., Goffin, B., & De Winter, G. (2024). Impact of mixing and shaking on mRNA-LNP drug product quality characteristics. *Scientific reports*, *14*(1), 19590. https://doi.org/10.1038/s41598-024-70680-4

Maugeri, M., Nawaz, M., Papadimitriou, A., Angerfors, A., Camponeschi, A., Na, M., Hölttä, M., Skantze, P., Johansson, S., Sundqvist, M., Lindquist, J., Kjellman, T., Mårtensson, I.-L., Jin, T., Sunnerhagen, P., Östman, S., Lindfors, L., & Valadi, H. (2019). Linkage between endosomal escape of LNP-mRNA and loading into EVs for transport to other cells. *Nature Communications*, *10*(1), 4333. https://doi.org/10.1038/s41467-019-12275-6

Mendonça, M. C. P., Kont, A., Kowalski, P. S., & O'Driscoll, C. M. (2023). Design of lipid-based nanoparticles for delivery of therapeutic nucleic acids. *Drug discovery today*, *28*(3), 103505. https://doi.org/https://doi.org/10.1016/j.drudis.2023.103505

Meredith Packer , Dipendra Gyawali, Serenus Hua , Gabor Butora , & Mercer, G. J. (2022). *Lipid Nanoparticle Compositions and Methods of Formulating the Same* (United States Patent No. US011524023B2). U. P. Office. https://patentimages.storage.googleapis.com/41/fc/0f/3fde60367c88fa/US11524023.pdf

Miao, L., Lin, J., Huang, Y., Li, L., Delcassian, D., Ge, Y., Shi, Y., & Anderson, D. G. (2020). Synergistic lipid compositions for albumin receptor mediated delivery of mRNA to the liver. *Nature Communications*, *11*(1), 2424. https://doi.org/10.1038/s41467-020-16248-y

Milano, G., Gal, J., Creisson, A., & Chamorey, E. (2021). Myocarditis and COVID-19 mRNA vaccines: a mechanistic hypothesis involving dsRNA. *Future Virol*. https://doi.org/10.2217/fvl-2021-0280

Mizuno, T., Takahashi, R., Kamiyama, T., Suzuki, A., & Suzuki, M. (2022). Neuroleptic Malignant Syndrome with Adrenal Insufficiency After BNT162b2 COVID-19 Vaccination in a Man Taking Valproate: A Case Report. *Am J Case Rep*, *23*, e936217. https://doi.org/10.12659/ajcr.936217

Moderna. (2022). *Moderna Science and Technology Day 2022*. https://s29.q4cdn.com/435878511/files/doc_presentations/2022/05/Science-Day-2022-Master-Slides-FINAL-(05.17_7am).pdf. Retrieved February 28, 2023 from

Mui, B. L., Tam, Y. K., Jayaraman, M., Ansell, S. M., Du, X., Tam, Y. Y., Lin, P. J., Chen, S., Narayanannair, J. K., Rajeev, K. G., Manoharan, M., Akinc, A., Maier, M. A., Cullis, P., Madden, T. D., & Hope, M. J. (2013). Influence of Polyethylene Glycol Lipid Desorption Rates on Pharmacokinetics and Pharmacodynamics of siRNA Lipid Nanoparticles. *Molecular therapy. Nucleic acids*, *2*(12), e139. https://doi.org/10.1038/mtna.2013.66

Mukai, H., Ogawa, K., Kato, N., & Kawakami, S. (2022). Recent advances in lipid nanoparticles for delivery of nucleic acid, mRNA, and gene editing-based therapeutics. *Drug Metabolism and Pharmacokinetics*, *44*, 100450. https://doi.org/https://doi.org/10.1016/j.dmpk.2022.100450

Müller, J. A., Schäffler, N., Kellerer, T., Schwake, G., Ligon, T. S., & Rädler, J. O. (2024). Kinetics of RNA-LNP delivery and protein expression. *European Journal of Pharmaceutics and Biopharmaceutics*, *197*, 114222. https://doi.org/https://doi.org/10.1016/j.ejpb.2024.114222

Münter, R., Larsen, J. B., & Andresen, T. L. (2024). The vast majority of nucleic acid-loaded lipid nanoparticles contain cargo. *Journal of Colloid and Interface Science*, *674*, 139-144. https://doi.org/https://doi.org/10.1016/j.jcis.2024.06.158

Naasani, I. (2022). Establishing the Pharmacokinetics of Genetic Vaccines is Essential for Maximising their Safety and Efficacy. *Clin Pharmacokinet*, *61*(7), 921-927. https://doi.org/10.1007/s40262-022-01149-8

Neves, A. R., Queiroz, J. F., Costa Lima, S. A., Figueiredo, F., Fernandes, R., & Reis, S. (2016). Cellular uptake and transcytosis of lipid-based nanoparticles across the intestinal barrier: Relevance for oral drug delivery. *Journal of Colloid and Interface Science*, *463*, 258-265. https://doi.org/https://doi.org/10.1016/j.jcis.2015.10.057

Ngo, W., Ahmed, S., Blackadar, C., Bussin, B., Ji, Q., Mladjenovic, S. M., Sepahi, Z., & Chan, W. C. W. (2022). Why nanoparticles prefer liver macrophage cell uptake in vivo. *Adv Drug Deliv Rev*, *185*, 114238. https://doi.org/10.1016/j.addr.2022.114238

Nogueira, S. S., Samaridou, E., Simon, J., Frank, S., Beck-Broichsitter, M., & Mehta, A. (2024). Analytical techniques for the characterization of nanoparticles for mRNA delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *198*, 114235. https://doi.org/https://doi.org/10.1016/j.ejpb.2024.114235

Obeng, R. C., Escobar, D. J., Vadasz, B., Zheng, W., Ju, J. Y., Booth, A. L., Yang, G. Y., Al Diffalha, S., Dhall, D., Westerhoff, M., & Xue, Y. (2025). Histologic Features of Liver Injury Associated With SARS-CoV-2 Messenger RNA Vaccines. *Arch Pathol Lab Med*, *149*(6), 556-560. https://doi.org/10.5858/arpa.2024-0095-OA

Omo-Lamai, S., Wang, Y., Patel, M. N., Milosavljevic, A., Zuschlag, D., Poddar, S., Wu, J., Wang, L., Dong, F., Espy, C., Majumder, A., Essien, E. O., Shen, M., Channer, B., Papp, T. E., Tobin, M., Maheshwari, R., Jeong, S., Patel, S., . . . Brenner, J. S. (2025). Limiting endosomal damage sensing reduces inflammation triggered by lipid nanoparticle endosomal escape. *Nat Nanotechnol*, *20*(9), 1285-1297. https://doi.org/10.1038/s41565-025-01974-5

Oude Blenke, E., Örnskov, E., Schöneich, C., Nilsson, G. A., Volkin, D. B., Mastrobattista, E., Almarsson, Ö., & Crommelin, D. J. A. (2023). The Storage and In-Use Stability of mRNA Vaccines and Therapeutics: Not A Cold Case. *Journal of Pharmaceutical Sciences*, *112*(2), 386-403. https://doi.org/10.1016/j.xphs.2022.11.001

Packer, M., Gyawali, D., Yerabolu, R., Schariter, J., & White, P. (2021). A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems. *Nat Commun*, *12*(1), 6777. https://doi.org/10.1038/s41467-021-26926-0

Paramasivam, P., Franke, C., Stöter, M., Höijer, A., Bartesaghi, S., Sabirsh, A., Lindfors, L., Arteta, M. Y., Dahlén, A., Bak, A., Andersson, S., Kalaidzidis, Y., Bickle, M., & Zerial, M. (2021). Endosomal escape of delivered mRNA from endosomal recycling tubules visualized at the nanoscale. *Journal of Cell Biology*, *221*(2). https://doi.org/10.1083/jcb.202110137

Parot, J., Mehn, D., Jankevics, H., Markova, N., Carboni, M., Olaisen, C., Hoel, A. D., Sigfúsdóttir, M. S., Meier, F., Drexel, R., Vella, G., McDonagh, B., Hansen, T., Bui, H., Klinkenberg, G., Visnes, T., Gioria, S., Urban-Lopez, P., Prina-Mello, A., . . . Calzolai, L. (2024). Quality assessment of LNP-RNA therapeutics with orthogonal analytical techniques. *Journal of Controlled Release*, *367*, 385-401. https://doi.org/https://doi.org/10.1016/j.jconrel.2024.01.037

Pateev, I., Seregina, K., Ivanov, R., & Reshetnikov, V. (2023). Biodistribution of RNA Vaccines and of Their Products: Evidence from Human and Animal Studies. *Biomedicines*, *12*(1). https://doi.org/10.3390/biomedicines12010059

Patel, P., Ibrahim, N. M., & Cheng, K. (2021). The Importance of Apparent pKa in the Development of Nanoparticles Encapsulating siRNA and mRNA. *Trends in Pharmacological Sciences*, *42*(6), 448-460. https://doi.org/10.1016/j.tips.2021.03.002

Paunovska, K., Da Silva Sanchez, A. J., Lokugamage, M. P., Loughrey, D., Echeverri, E. S., Cristian, A., Hatit, M. Z. C., Santangelo, P. J., Zhao, K., & Dahlman, J. E. (2022). The Extent to Which Lipid Nanoparticles Require Apolipoprotein E and Low-Density Lipoprotein Receptor for Delivery Changes with Ionizable Lipid Structure. *Nano Lett*, *22*(24), 10025-10033. https://doi.org/10.1021/acs.nanolett.2c03741

Pavlin, N., Bavčar, M., Kovačič, T., Kašček, T., Celjar, A. M., Bergoč, I., Livk, A. G., & Štrancar, A. (2025). Analysis of lipid nanoparticles using two-dimensional chromatography: Simultaneous determination of encapsulation efficiency, nucleic acid integrity, and size of LNP formulations. *Journal of Chromatography B*, *1265*, 124751. https://doi.org/https://doi.org/10.1016/j.jchromb.2025.124751

Peden, K. (2022, October 11, 2022). Considerations for the Quality, Safety and Efficacy of Prophylactic Lipid Nanoparticle mRNA Vaccines. Public Workshop on FDA Guidance to Industry on Nanomaterials.

Petersen, D. M. S., Weiss, R. M., Hajj, K. A., Yerneni, S. S., Chaudhary, N., Newby, A. N., Arral, M. L., & Whitehead, K. A. (2024). Branched-Tail Lipid Nanoparticles for Intravenous mRNA Delivery to Lung Immune, Endothelial, and Alveolar Cells in Mice. *Advanced Healthcare Materials*, *13*(22), 2400225. https://doi.org/https://doi.org/10.1002/adhm.202400225

Pilkington, E. H., Suys, E. J. A., Trevaskis, N. L., Wheatley, A. K., Zukancic, D., Algarni, A., Al-Wassiti, H., Davis, T. P., Pouton, C. W., Kent, S. J., & Truong, N. P. (2021). From influenza to COVID-19: Lipid nanoparticle mRNA vaccines at the frontiers of infectious diseases. *Acta Biomater*, *131*, 16-40. https://doi.org/10.1016/j.actbio.2021.06.023

Quick, J., Santos, N. D., Cheng, M. H. Y., Chander, N., Brimacombe, C. A., Kulkarni, J., van der Meel, R., Tam, Y. Y. C., Witzigmann, D., & Cullis, P. R. (2022). Lipid nanoparticles to silence androgen receptor variants for prostate cancer therapy. *J Control Release*, *349*, 174-183. https://doi.org/10.1016/j.jconrel.2022.06.051

Ren, L., Zhao, Z., Chao, Y., Yu, P., Mei, Z., Du, B., & Cheng, Y. (2025). Optimization of Lipid Nanoparticles with Robust Efficiency for the Delivery of Protein Therapeutics to Augment Cancer Immunotherapy. *Advanced Science*, *12*(17), 2500844. https://doi.org/https://doi.org/10.1002/advs.202500844

Ren, Y., Lin, L., Abdallah, M., Zhu, X., Liu, H., Fabb, S. A., Payne, T. J., Pouton, C. W., Johnston, A. P. R., & Trevaskis, N. L. (2025). Impact of ionizable lipid type on the pharmacokinetics and biodistribution of mRNA-lipid nanoparticles after intravenous and subcutaneous injection. *Journal of Controlled Release*, *384*, 113945. https://doi.org/https://doi.org/10.1016/j.jconrel.2025.113945

Renzi, S., Digiacomo, L., Pozzi, D., Quagliarini, E., Vulpis, E., Giuli, M. V., Mancusi, A., Natiello, B., Pignataro, M. G., Canettieri, G., Di Magno, L., Pesce, L., De Lorenzi, V., Ghignoli, S., Loconte, L., Montone, C. M., Laura Capriotti, A., Laganà, A., Nicoletti, C., . . . Caracciolo, G. (2024). Structuring lipid nanoparticles, DNA, and protein corona into stealth bionanoarchitectures for in vivo gene delivery. *Nature Communications*, *15*(1), 9119. https://doi.org/10.1038/s41467-024-53569-8

Rezaei, S., Blick, E. E., Mineart, K. P., & Kelley, E. G. (2025). Chapter Three - Linking chemical degradation and physical instability of lipid vesicles. In A. Iglič, M. Rappolt, & P. Losada-Pérez (Eds.), *Advances in Biomembranes and Lipid Self-Assembly* (Vol. 41, pp. 47-64). Academic Press. https://doi.org/https://doi.org/10.1016/bs.abl.2025.05.001

Rigby, R. E., & Rehwinkel, J. (2015). RNA degradation in antiviral immunity and autoimmunity. *Trends in Immunology*, *36*(3), 179-188. https://doi.org/10.1016/j.it.2015.02.001

Sabnis, S., Kumarasinghe, E. S., Salerno, T., Mihai, C., Ketova, T., Senn, J. J., Lynn, A., Bulychev, A., McFadyen, I., Chan, J., Almarsson, Ö., Stanton, M. G., & Benenato, K. E. (2018). A Novel Amino Lipid Series for mRNA Delivery: Improved Endosomal Escape and Sustained

Pharmacology and Safety in Non-human Primates. *Mol Ther*, *26*(6), 1509-1519. https://doi.org/10.1016/j.ymthe.2018.03.010

Sahay, G., Querbes, W., Alabi, C., Eltoukhy, A., Sarkar, S., Zurenko, C., Karagiannis, E., Love, K., Chen, D., Zoncu, R., Buganim, Y., Schroeder, A., Langer, R., & Anderson, D. G. (2013). Efficiency of siRNA delivery by lipid nanoparticles is limited by endocytic recycling. *Nat Biotechnol*, *31*(7), 653-658. https://doi.org/10.1038/nbt.2614

Sahin, U., Karikó, K., & Türeci, Ö. (2014). mRNA-based therapeutics — developing a new class of drugs. *Nature Reviews Drug Discovery*, *13*(10), 759-780. https://doi.org/10.1038/nrd4278

Sakurai, Y., Watanabe, H., Nishio, K., Hashimoto, K., Harada, A., Gomi, M., Suzuki, M., Oyama, R., Handa, T., Sato, R., Takeuchi, H., Taira, R., Tezuka, K., Tange, K., Nakai, Y., Akita, H., & Uchida, Y. (2022). pH-Responsive Lipid Nanoparticles Achieve Efficient mRNA Transfection in Brain Capillary Endothelial Cells. *Pharmaceutics*, *14*(8). https://doi.org/10.3390/pharmaceutics14081560

Sanyal, G., Särnefält, A., & Kumar, A. (2021). Considerations for bioanalytical characterization and batch release of COVID-19 vaccines. *NPJ Vaccines*, *6*(1), 53. https://doi.org/10.1038/s41541-021-00317-4

Schlich, M., Palomba, R., Costabile, G., Mizrahy, S., Pannuzzo, M., Peer, D., & Decuzzi, P. (2021). Cytosolic delivery of nucleic acids: The case of ionizable lipid nanoparticles. *Bioengineering & Translational Medicine*, *6*(2), e10213. https://doi.org/https://doi.org/10.1002/btm2.10213

Schober, G. B., Story, S., & Arya, D. P. (2024). A careful look at lipid nanoparticle characterization: analysis of benchmark formulations for encapsulation of RNA cargo size gradient. *Scientific reports*, *14*(1), 2403. https://doi.org/10.1038/s41598-024-52685-1

Schoenmaker, L., Witzigmann, D., Kulkarni, J. A., Verbeke, R., Kersten, G., Jiskoot, W., & Crommelin, D. J. A. (2021). mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. *International Journal of Pharmaceutics*, *601*, 120586. https://doi.org/https://doi.org/10.1016/j.ijpharm.2021.120586

Sebastiani, F., Yanez Arteta, M., Lerche, M., Porcar, L., Lang, C., Bragg, R. A., Elmore, C. S., Krishnamurthy, V. R., Russell, R. A., Darwish, T., Pichler, H., Waldie, S., Moulin, M., Haertlein, M., Forsyth, V. T., Lindfors, L., & Cárdenas, M. (2021). Apolipoprotein E Binding Drives Structural and Compositional Rearrangement of mRNA-Containing Lipid Nanoparticles. *ACS Nano*, *15*(4), 6709-6722. https://doi.org/10.1021/acsnano.0c10064

Sengottiyan, S., Mikolajczyk, A., Jagiełło, K., Swirog, M., & Puzyn, T. (2023). Core, Coating, or Corona? The Importance of Considering Protein Coronas in nano-QSPR Modeling of Zeta Potential. *ACS Nano*, *17*(3), 1989-1997. https://doi.org/10.1021/acsnano.2c06977

Sfera, A., Hazan, S., Anton, J. J., Sfera, D. O., Andronescu, C. V., Sasannia, S., Rahman, L., & Kozlakidis, Z. (2022). Psychotropic drugs interaction with the lipid nanoparticle of COVID-19 mRNA therapeutics. *Frontiers in pharmacology*, *13*, 995481-995481. https://doi.org/10.3389/fphar.2022.995481

Simon, C. G., Borgos, S. E., Calzolai, L., Nelson, B. C., Parot, J., Petersen, E. J., Roesslein, M., Xu, X., & Caputo, F. (2023). Orthogonal and complementary measurements of properties of drug products containing nanomaterials. *Journal of Controlled Release*, *354*, 120-127. https://doi.org/https://doi.org/10.1016/j.jconrel.2022.12.049

Simonsen, J. B. (2024). A perspective on bleb and empty LNP structures. *J Control Release*, *373*, 952-961. https://doi.org/10.1016/j.jconrel.2024.07.046

Song, J., Su, D., Wu, H., & Guo, J. (2025). Implications of Anaphylaxis Following mRNA-LNP Vaccines: It Is Urgent to Eliminate PEG and Find Alternatives. *Pharmaceutics*, *17*(6), 798. https://www.mdpi.com/1999-4923/17/6/798

Sousa de Almeida, M., Susnik, E., Drasler, B., Taladriz-Blanco, P., Petri-Fink, A., & Rothen-Rutishauser, B. (2021). Understanding nanoparticle endocytosis to improve targeting strategies in nanomedicine. *Chem Soc Rev*, *50*(9), 5397-5434. https://doi.org/10.1039/d0cs01127d

Sun, Y., Zhou, Y., Rehman, M., Wang, Y.-F., & Guo, S. (2024). Protein Corona of Nanoparticles: Isolation and Analysis. *Chem & Bio Engineering*, *1*(9), 757-772. https://doi.org/10.1021/cbe.4c00105

Swingle, K. L., Safford, H. C., Geisler, H. C., Hamilton, A. G., Thatte, A. S., Billingsley, M. M., Joseph, R. A., Mrksich, K., Padilla, M. S., Ghalsasi, A. A., Alameh, M.-G., Weissman, D., & Mitchell, M. J. (2023). Ionizable Lipid Nanoparticles for In Vivo mRNA Delivery to the Placenta during Pregnancy. *Journal of the American Chemical Society*, *145*(8), 4691-4706. https://doi.org/10.1021/jacs.2c12893

Szebeni, J., Simberg, D., González-Fernández, Á., Barenholz, Y., & Dobrovolskaia, M. A. (2018). Roadmap and strategy for overcoming infusion reactions to nanomedicines. *Nature Nanotechnology*, *13*(12), 1100-1108. https://doi.org/10.1038/s41565-018-0273-1

Tenchov, R., Bird, R., Curtze, A. E., & Zhou, Q. (2021). Lipid Nanoparticles─From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement. *ACS Nano*, *15*(11), 16982-17015. https://doi.org/10.1021/acsnano.1c04996

TherapeuticGoodsAdministration. (2021). *Nonclinical Evaluation Report BNT162b2 [mRNA] COVID-19 vaccine (COMIRNATY)*. https://www.tga.gov.au/sites/default/files/foi-2389-06.pdf: Department of Health and Aged Care Retrieved from https://www.tga.gov.au/sites/default/files/foi-2389-06.pdf

Thiemicke, A., & Neuert, G. (2023). Rate thresholds in cell signaling have functional and phenotypic consequences in non-linear time-dependent environments [Review]. *Frontiers in Cell and Developmental Biology*, *Volume 11 - 2023*. https://doi.org/10.3389/fcell.2023.1124874

Thompson, D., Delorme, C. M., White, R. F., & Honer, W. G. (2021). Elevated clozapine levels and toxic effects after SARS-CoV-2 vaccination. *Journal of psychiatry & neuroscience : JPN*, *46*(2), E210-E211. https://doi.org/10.1503/jpn.210027

Tomihari, A., Kiyota, M., Matsuura, A., & Itakura, E. (2023). Alpha 2-macroglobulin acts as a clearance factor in the lysosomal degradation of extracellular misfolded proteins. *Scientific reports*, *13*(1), 4680. https://doi.org/10.1038/s41598-023-31104-x

UnitedStatesPharmacopeia. (2024). Analytical Procedures for Quality of mRNA Vaccines and Therapeutics (Draft Guidelines: 3rd Edition). In. https://go.usp.org/mRNAVaccineQuality: USP-NF.

USFDA. (2020). *Pfizer-BioNTech COVID-19 Vaccine VRBPAC Briefing Document*. https://www.fda.gov/media/144246/download: US Health and Human Services Retrieved from https://www.fda.gov/media/144246/download

USFDA. (2021). *Letter to Pfizer: Children's vaccination, authorization of formulation change*. https://cacmap.fda.gov/media/150386/download: US Food and Drug Administration Retrieved from https://himasanpablo.com/wp-content/uploads/2021/11/Pfizer-BioNTech-COVID-19-Vaccine-Letter-of-Authorization-Rev.-10-29-2021.pdf

USFDA. (2022). *Drug Products Including Biological Products, which Contain Nanomaterials*. Silver Springs, MD Retrieved from https://www.regulations.gov/document/FDA-2017-D-0759-0017

Veerman, S. R. T., Bogers, J., Cohen, D., & Schulte, P. F. J. (2022). COVID-19: Risks, Complications, and Monitoring in Patients on Clozapine. *Pharmacopsychiatry*, *55*(1), 48-56. https://doi.org/10.1055/a-1562-2521

Vervaeke, P., Borgos, S. E., Sanders, N. N., & Combes, F. (2022). Regulatory guidelines and preclinical tools to study the biodistribution of RNA therapeutics. *Advanced Drug Delivery Reviews*, *184*, 114236. https://doi.org/https://doi.org/10.1016/j.addr.2022.114236

Vijay, S., & Gujral, T. S. (2020). Non-linear Deep Neural Network for Rapid and Accurate Prediction of Phenotypic Responses to Kinase Inhibitors. *iScience*, *23*(5). https://doi.org/10.1016/j.isci.2020.101129

Villemure, S., Trenaman, S. C., & Goralski, K. B. (2023). The impact of COVID-19 infection on cytochrome P450 3A4-mediated drug metabolism and drug interactions. *Expert Opinion on Drug Metabolism & Toxicology*, *19*(6), 329-332. https://doi.org/10.1080/17425255.2023.2228680

Voke, E., Arral, M., Squire, H. J., Lin, T. J., Coreas, R., Lui, A., Iavarone, A. T., Pinals, R. L., Whitehead, K. A., & Landry, M. (2025). Protein corona formed on lipid nanoparticles compromises delivery efficiency of mRNA cargo. *bioRxiv*. https://doi.org/10.1101/2025.01.20.633942

Walczyk, D., Bombelli, F. B., Monopoli, M. P., Lynch, I., & Dawson, K. A. (2010). What the Cell "Sees" in Bionanoscience. *Journal of the American Chemical Society*, *132*(16), 5761-5768. https://doi.org/10.1021/ja910675v

Wang, J., Ding, Y., Chong, K., Cui, M., Cao, Z., Tang, C., Tian, Z., Hu, Y., Zhao, Y., & Jiang, S. (2024). Recent Advances in Lipid Nanoparticles and Their Safety Concerns for mRNA Delivery. *Vaccines (Basel)*, *12*(10). https://doi.org/10.3390/vaccines12101148

Webb, A. L. J., Welbourne, E. N., Evans, C. A., & Dickman, M. J. (2025). Characterisation and analysis of mRNA critical quality attributes using liquid chromatography based methods. *Journal of Chromatography A*, *1745*, 465724. https://doi.org/https://doi.org/10.1016/j.chroma.2025.465724

Wegler, C., Ölander, M., Wiśniewski, J. R., Lundquist, P., Zettl, K., Åsberg, A., Hjelmesæth, J., Andersson, T. B., & Artursson, P. (2019). Global variability analysis of mRNA and protein concentrations across and within human tissues. *NAR Genomics and Bioinformatics*, *2*(1). https://doi.org/10.1093/nargab/lqz010

WorldHealthOrganization. (2005). *WHO Guidelines on Non-Clinical Evaluation of Vaccines TRS No 927*. (WHO TRS No 927). https://www.who.int/publications/m/item/nonclinical-evaluation-of-vaccines-annex-1-trs-no-927: World Health Organization

Wu, P., Zhang, B., Ocansey, D. K. W., Xu, W., & Qian, H. (2021). Extracellular vesicles: A bright star of nanomedicine. *Biomaterials*, *269*, 120467. https://doi.org/https://doi.org/10.1016/j.biomaterials.2020.120467

Yamamoto, K., Scilabra, S. D., Bonelli, S., Jensen, A., Scavenius, C., Enghild, J. J., & Strickland, D. K. (2024). Novel insights into the multifaceted and tissue-specific roles of the endocytic receptor LRP1. *The Journal of biological chemistry*, *300*(8), 107521. https://doi.org/10.1016/j.jbc.2024.107521

Yang, L., Gong, L., Wang, P., Zhao, X., Zhao, F., Zhang, Z., Li, Y., & Huang, W. (2022). Recent Advances in Lipid Nanoparticles for Delivery of mRNA. *Pharmaceutics*, *14*(12). https://doi.org/10.3390/pharmaceutics14122682

Younis, M. A., Sato, Y., Elewa, Y. H. A., Kon, Y., & Harashima, H. (2023). Self-homing nanocarriers for mRNA delivery to the activated hepatic stellate cells in liver fibrosis. *Journal of Controlled Release*, *353*, 685-698. https://doi.org/https://doi.org/10.1016/j.jconrel.2022.12.020

Yuan, Z., Yan, R., Fu, Z., Wu, T., & Ren, C. (2024). Impact of physicochemical properties on biological effects of lipid nanoparticles: Are they completely safe. *Science of The Total Environment*, *927*, 172240. https://doi.org/https://doi.org/10.1016/j.scitotenv.2024.172240

Żak, M. M., Kaur, K., Yoo, J., Kurian, A. A., Adjmi, M., Mainkar, G., Yoon, S., & Zangi, L. (2023). Modified mRNA Formulation and Stability for Cardiac and Skeletal Muscle Delivery. *Pharmaceutics*, *15*(9). https://doi.org/10.3390/pharmaceutics15092176

Zech, T., Ejsing, C. S., Gaus, K., de Wet, B., Shevchenko, A., Simons, K., & Harder, T. (2009). Accumulation of raft lipids in T-cell plasma membrane domains engaged in TCR signalling. *The EMBO Journal*, *28*(5), 466-476. https://doi.org/https://doi.org/10.1038/emboj.2009.6

Zelkoski, A. E., Lu, Z., Sukumar, G., Dalgard, C., Said, H., Alameh, M.-G., Mitre, E., & Malloy, A. M. W. (2025). Ionizable lipid nanoparticles of mRNA vaccines elicit NF-κB and IRF responses through toll-like receptor 4. *NPJ Vaccines*, *10*(1), 73. https://doi.org/10.1038/s41541-025-01124-x

Zhang, H., & Barz, M. (2025). Investigating the stability of RNA-lipid nanoparticles in biological fluids: Unveiling its crucial role for understanding LNP performance. *Journal of Controlled Release*, *381*, 113559. https://doi.org/https://doi.org/10.1016/j.jconrel.2025.02.055

Zhang, L., More, K. R., Ojha, A., Jackson, C. B., Quinlan, B. D., Li, H., He, W., Farzan, M., Pardi, N., & Choe, H. (2023). Effect of mRNA-LNP components of two globally-marketed COVID-19 vaccines on efficacy and stability. *NPJ Vaccines*, *8*(1), 156. https://doi.org/10.1038/s41541-023-00751-6

Zhang, L., Seow, B. Y. L., Bae, K. H., Zhang, Y., Liao, K.-C., Wan, Y., & Yang, Y. Y. (2025). Role of PEGylated lipid in lipid nanoparticle formulation for in vitro and in vivo delivery of mRNA

vaccines. *Journal of Controlled Release*, *380*, 108-124. https://doi.org/https://doi.org/10.1016/j.jconrel.2025.01.071

Zhang, T., Yin, H., Li, Y., Yang, H., Ge, K., Zhang, J., Yuan, Q., Dai, X., Naeem, A., Weng, Y., Huang, Y., & Liang, X.-J. (2024). Optimized lipid nanoparticles (LNPs) for organ-selective nucleic acids delivery in vivo. *iScience*, *27*(6), 109804. https://doi.org/https://doi.org/10.1016/j.isci.2024.109804

Zhichang Yang, P. B., Sahana Mollah, Robert Proos, Jonathan Le Huray. (2023). *Structural characterization of the cationic lipid nanoparticle component, ALC-0315, and its impurities using electronactivated dissociation (EAD)-based MS/MS fragmentation*. https://sciex.com/tech-notes/biopharma/structural-characterization-of-the-cationic-lipid-nanoparticle-c